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# Einfluss einer maternalen Supplementation mit essentiellen Fettsäuren und konjugierter Linolsäure auf den Fettsäurestatus sowie den Energiestoffwechsel und die Entwicklung neugeborener Kälber

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M.Sc. Katrin Lena Uken
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Dekan: Prof. Dr. Karl H. Mühling

1. Berichterstatter: PD Dr. Harald M. Hammon
2. Berichterstatter: Prof. Dr. Siegfried J. Wolffram

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# Einfluss einer maternalen Supplementation mit essentiellen Fettsäuren und konjugierter Linolsäure auf den Fettsäurestatus sowie den Energiestoffwechsel und die Entwicklung neugeborener Kälber

[Impact of maternal supplementation with essential fatty acids and conjugated linoleic acid on the fatty acid status as well as the energy metabolism and development of neonatal calves]

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# Abbreviations

| | |
|---|---|
| ADG | average daily gain |
| ALA | α-linolenic acid |
| ANOVA | analysis of variance |
| APE | atom percent excess |
| ARA | arachidonic acid |
| AUC | area under the curve |
| BHB | β-hydroxybutyrate |
| BW | body weight |
| CE | cholesterol esters |
| CD | conjuagted diene |
| CLA | conjugated linoleic acid |
| CTRL | control group (dams received coconut oil) |
| CV | coefficient of variation |
| d | day |
| DHA | docosahexaenoic acid |
| DIM | days in milk |
| DM | dry matter |
| DPA | docosapentaenoic acid |
| EDTA | ethylenediaminetetraacetic acid |
| EFA | essential fatty acids |
| ELISA | enzyme-linked immunosorbent assay |
| EPA | eicosapentaenoic acid |
| FA | fatty acid |
| FFA | free fatty acid |
| FPU | first-pass uptake |
| GH | growth hormone |
| GPR | G-protein coupled surface receptors |
| h | hour |
| HPLC | high performance liquid chromatography |
| Ig | immunoglobulin |
| IGF | insulin-like growth factor |

| | |
|---|---|
| IGFBP | insulin-like growth factor binding proteins |
| IU | international units |
| LA | linoleic acid |
| LSM | least squares mean |
| min | minute |
| MPE | mole percent excess |
| NEFA | non-esterified fatty acids |
| $PGE_2$ | prostaglandin E2 |
| PGF2α | prostaglandin F2 |
| PPAR | peroxisome proliferator-activated receptors |
| PUFA | polyunsaturated fatty acids |
| Ra | rate of appearance |
| RIA | radioimmunoassay |
| PL | phospholipids |
| RQUICKI | revised quantitative insulin sensitivity check index |
| SE | standard error |
| TG | triglycerides |
| TMR | total mixed ration |
| wk | week |

# General introduction

## General introduction

The term essential fatty acids (EFA) summarizes the two fatty acids linoleic acid (LA, n-6) and α-linolenic acid (ALA, n-3), which are indispensable for the organism, serving as membrane components, ligands of transcription factors, and precursors for lipid mediators (Burr and Burr, 1930; Burr et al., 1932; Innis, 2005). Especially for the developing fetus, these fatty acids are important due to their critical role in cognitive development (Koletzko et al., 2008). However, particularly the EFA supply via the bovine placenta is assumed to be low, resulting in a poor EFA status in the neonate (Noble et al., 1978). The availability of n-3 fatty acids for calves might be further limited due to the common replacement of pasture by corn silage-based diets, which contain less ALA but more LA than pasture (Ferlay et al., 2006). Moreover, feeding corn silage-based diets reduces the synthesis of conjugated linoleic acids (CLA) compared to pasture (Couvreur et al., 2006). Whether an altered maternal ALA and CLA status can be transferred to the calf is still unclear, though.

Previous studies indicate that an increased dietary intake of ALA or both EFA can affect the metabolism, including modulations of glucose, urea, and insulin-like growth factor-I levels in plasma and serum of calves (Hill et al., 2009; Garcia et al., 2014). Likewise, CLA supplementation was previously associated with modulations of the metabolism, such as body fat reducing effects and alleviation of hyperinsulinemia (Park et al., 1997; Nagao et al., 2003). Furthermore, an altered fatty acid supply might change aspects of the development as suggested by improved weight gain in calves supplemented with EFA and a changed intestinal morphometry in piglets from sows supplemented with n-3 fatty acids (Jenkins and Kramer, 1986; Boudry et al., 2009; Garcia et al., 2014). However, it is unknown whether the metabolism and development of neonatal calves can also be modulated by an altered maternal EFA and CLA supply during gestation and via the intake of colostrum and milk.

To address these knowledge gaps, the present thesis aimed to elucidate the impact of maternal EFA and CLA supplementation on the fatty acid status of neonatal calves and on

aspects of their metabolism and development. For this purpose, plasma fatty acids of calves born from dams, which received EFA, CLA, or a combination of both, were analyzed after birth and after intake of colostrum and transition milk from their dams during the first five days of life. Aspects of the metabolism and development were investigated by measurement of various plasma metabolites and hormones related to energy metabolism and growth, determination of energy expenditure and first-pass uptake of glucose by stable isotope tracer technique as well as measurement of body weight, organ weight and intestinal morphometry.

This thesis is subdivided into four chapters. Chapter 1 introduces the importance of EFA and CLA supply for the neonatal fatty acid status and function as well as general aspects of the energy metabolism of neonatal calves. Chapter 2 addresses the impact of maternal supplementation with EFA, CLA or both on the fatty acid status and performance of neonatal calves and on the composition of colostrum and transition milk. Chapter 3 focuses on the modulation of the metabolism, organ weight, and intestinal morphometry in response to maternal supplementation with EFA, CLA or both. In Chapter 4, results from chapters 2 and 3 are generally discussed and put into context to present literature.

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# CHAPTER 1

## Literature overview

# Literature overview

## 1.1 Essential fatty acids and their mechanisms of action

The two fatty acids linoleic acid (LA; 18:2 *cis*-9, *cis*-12), which is an n-6 fatty acid, and α-linolenic acid (ALA; 18:3 *cis*-9, *cis*-12, *cis*-15), an n-3 fatty acid, are classified as essential fatty acids (EFA). Their essentiality was already described in the 1930s by Burr and Burr (1930; 1932), who observed that rats fed a fat free diet developed scaly skin, growth retardation and an unusually high water intake, which could be cured by gavages of corn oil and linseed oil. The authors concluded that both fatty acids cannot be synthesized sufficiently by warm blooded animals and thus need to be taken up via food. For different species, it is assumed that 1-2% of the total caloric intake needs to be taken up as LA to fulfill the minimum requirement (Holman, 1971; Jenkins and Kramer, 1986). However, the EFA requirement of ruminants is still unknown.

Although LA and ALA cannot be synthesized by the animal, they can be converted to long-chain derivatives by multiple elongation and desaturation reactions mainly occurring in the liver (Calder, 2012). The desaturation of both, n-3 and n-6 fatty acids, is catalyzed by Δ-5 and Δ-6 desaturase and the two fatty acids thus compete with each other for the formation of their longer-chain derivatives (Figure 1.1). As a consequence, the production of derivatives from one of the EFA is also determined by the intake of the other and the n-6:n-3 fatty acid ratio is an important determinant of derivative synthesis and thus of fatty acid action (Calder, 2012; Salehi and Ambrose, 2017). However, these reactions are assumed to be predominantly limited by the activity of Δ-6 desaturase (Calder, 2012), which has a higher affinity for desaturation of n-3 fatty acids (Geiger et al., 1993). Desaturation and elongation of ALA yields the n-3 long-chain fatty acids eicosapentaenoic acid (EPA, 20:5 *cis*-5, *cis*-8, *cis*-11), docosapentaenoic acid (DPA, 22:5 *cis*-7, *cis*-10, *cis*-13, *cis*-16, *cis*-19), and docosahexaenoic acid (DHA, 22:6 *cis*-4, *cis*-7, *cis*-10, *cis*-13, *cis*-16, *cis*-19) as well as other n-3 intermediates. From LA, for instance, the n-6 fatty acid arachidonic acid (ARA, 20:4 *cis*-5, *cis*-8, *cis*-11, *cis*-14) can be formed. Although

EFA possess some characteristics of very long-chain fatty acids, they have a low biological potency and their actions are assumed to be attained predominantly from the conversion to their longer chain derivatives (Calder, 2012).

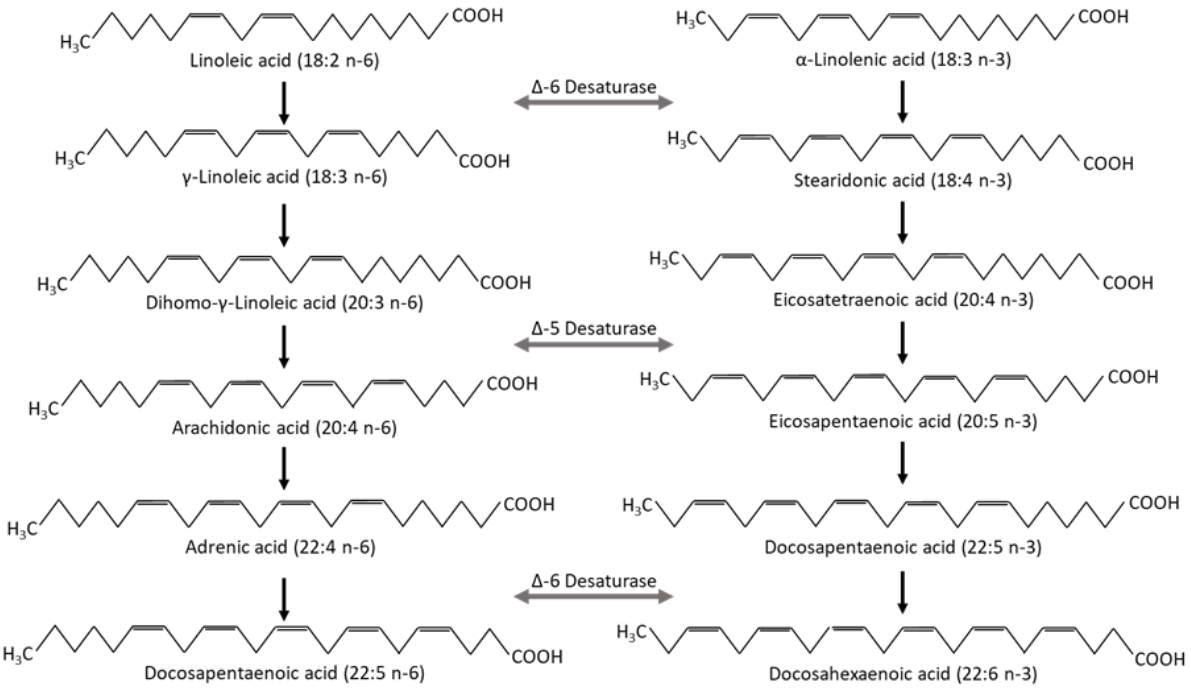


**Figure 1.1: Overview of major pathways of essential fatty acid biosynthesis. Adapted from Innis (2005) and Calder (2012).**

There are several mechanisms by which fatty acids can induce effects on the organism. One of these mechanisms is the binding of fatty acids to cell surface and intracellular receptors and sensors modulating gene expression (Calder, 2012). These receptors include peroxisome proliferator-activated receptors (PPAR) transcription factors that are activated by ligands such as polyunsaturated fatty acids (PUFA) and eicosanoid mediators (Forman et al., 1997). The PPAR are involved in controlling β-oxidation, lipoprotein metabolism, fatty acid synthesis, adipose tissue development as well as insulin sensitivity (Schoonjans et al., 1996; Varga et al., 2011). For instance, activation of PPARα promotes the synthesis of enzymes that catalyze hepatic fatty acid oxidation resulting in a reduction of blood triglycerides, and activation of

PPARγ induces the expression of glucose transporter-4 and lipoprotein lipase, which promote the uptake of glucose and fatty acids into adipocytes and muscle cells (Clarke, 2000). Both PPAR are known to bind n-3 fatty acids such as EPA (Deckelbaum et al. 2006; Hardwick et al., 2013). Also, G-protein coupled surface receptors (GPR) can bind long-chain fatty acids, such as EPA and DHA, which were shown to induce insulin sensitizing effects mediated by binding to GPR120 (Oh et al., 2010). However, actions of fatty acids are not only facilitated by fatty acid receptors, various effects can also be exerted by altered physical properties of the membrane due to changes of the membrane fatty acid composition in response to fatty acid availability (Calder, 2012). The fatty acid composition in membrane phospholipids determines the mobility and function of membrane proteins, membrane fluidity, and the formation of lipid rafts (Murphy, 1990; Calder, 2012).

Moreover, membrane phospholipids provide a source for n-3 and n-6 PUFA, which can serve as substrates for the synthesis of lipid mediators (Calder, 2012). These lipid mediators include eicosanoids, such as prostaglandins, leukotrienes, and thromboxanes, which take part in controlling inflammation, immunity and the cell metabolism (Calder, 2001; Calder, 2012; Hardwick et al., 2013). Due to its high abundance in membranes, the n-6 fatty acid ARA is the major substrate for eicosanoid synthesis (Calder, 2001). Although both pro- and anti-inflammatory eicosanoids can be produced from n-6 fatty acids, an excessive production of n-6 fatty acid-based eicosanoids might be involved in disease processes (Serhan and Petasis; 2011, Calder, 2012). Moreover, the metabolism might be affected by an increased n-6:n-3 fatty acid ratio. For instance, a high intake of n-6 and a simultaneously low intake of n-3 fatty acids is associated with an increased production of prostaglandin $E_2$ ($PGE_2$), which enhances the uptake of triglycerides in adipocytes and is associated with diabetes and obesity (Patterson et al., 2012; Hardwick et al., 2013). However, the availability of ARA for eicosanoid production and ARA metabolism can be inhibited by an enhanced supply of n-3 fatty acids (Calder, 2012). Furthermore, the n-3 fatty acids EPA and DHA can serve as precursors for lipid mediators,

which are less potent or have anti-inflammatory, pro-resolving, or cytoprotective properties (Serhan and Petasis; 2011, Calder, 2012). Thus, actions of EFA can be mediated via multiple pathways, which might affect various aspects of the organism such as the metabolism and immune system.

## 1.2 Conjugated linoleic acids and their mechanisms of action

The term, conjugated linoleic acids (CLA) summarizes octadecadienoic acids with conjugated double bounds, which include more than 28 different isomers with *cis*-*trans*, *trans*-*cis*, *cis*-*cis*, or *trans*-*trans* configurations (Bauman et al., 1999; Banni, 2002). However, especially *cis*-9, *trans*-11 and *trans*-10, *cis*-12 CLA are well-known isomers with *cis*-9, *trans*-11 CLA being the dominant isomer in milk fat and the *trans*-10, *cis*-12 isomer being in the focus of interest for its milk fat reducing properties (Parodi, 1977; Bauman et al., 1999). One source for CLA in ruminants is the incomplete biohydrogenation of dietary unsaturated fatty acids by ruminal microbes. The biohydrogenation of unsaturated fatty acids involves not only diverse bacteria but also various biochemical steps (Bauman et al., 1999). Biohydrogenation of *cis*-9, *cis*-12 containing fatty acids (LA, ALA and γ-linolenic acid) includes a series of isomerization and reduction steps which yield stearic acid (18:0) as end product (Figure 1.2). From these steps, *cis*-9, *trans*-11 CLA is produced as an intermediate from LA. In addition, vaccenic acid (18:1 *trans*-11) is synthesized as intermediate from LA, ALA and γ-linolenic acid (Bauman et al., 1999). As the reduction of vaccenic acid seems to be a rate limiting step in the biohydrogenation process, vaccenic acid accumulates in the rumen and can be readily absorbed (Bauman et al., 1999). Subsequently, *cis*-9, *trans*-11 CLA can be formed from vaccenic acid by endogenous synthesis, which is catalyzed by multiple enzymes including Δ-9 desaturase (Keeney, 1970; Bauman et al., 1999).

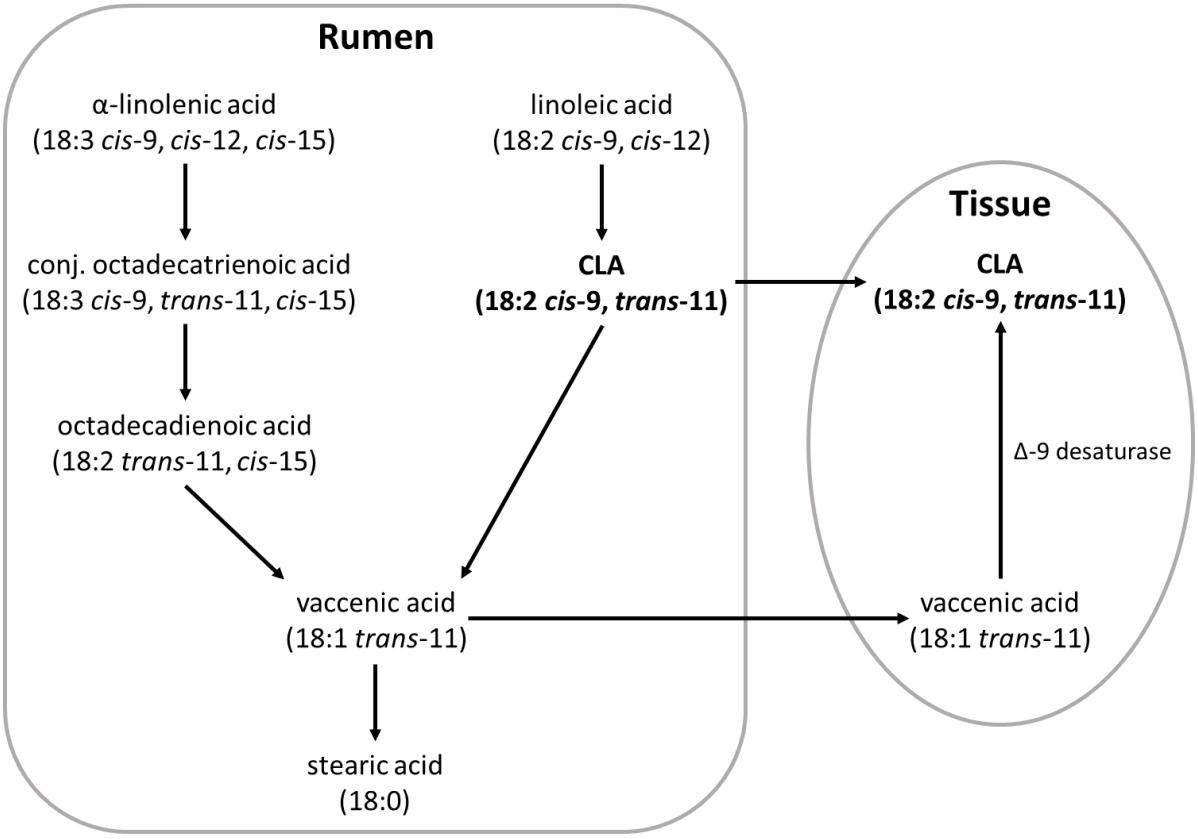


**Figure 1.2: Simplified scheme of predominant CLA synthesis pathways in the rumen and endogenous synthesis in tissues. Adapted from Griinari and Bauman (1999) and Bauman et al. (1999).**

In lactating ruminants, the mammary tissue is the predominant site of endogenous *cis*-9, *trans*-11 CLA synthesis, which is also the origin of the majority of CLA found in milk fat (Bauman et al., 1999; Griinari and Bauman, 1999). In growing ruminants, adipose tissue is regarded as main site of endogenous *cis*-9, *trans*-11 CLA synthesis (Whale, 1974; Bauman et al., 1999). Thus, not only the formation of CLA in the rumen but also the ruminal production of vaccenic acid and the activity of Δ-9 desaturase determine the CLA status in ruminants and products of ruminant origin (Bauman et al., 1999). Moreover, the composition of the produced CLA isomers can vary in response to the diet. For instance, low-fiber diets can promote the proportion of *trans*-10, *cis*-12 CLA in rumen digesta, which is assumed to be synthesized from

bacterial *cis*-9, *cis*-12 containing fatty acids via *cis*-9, *trans*-10 isomerase (Griinari and Bauman, 1999).

Subsequently, CLA might exert its effects on the organism in different ways. Due to its two double bonds, CLA is metabolized like LA including elongation, Δ-6 desaturation, and Δ-5 desaturation (Banni, 2002). The structure of the CLA metabolites resembles those produced from LA but the conjugated double bounds are maintained in CLA metabolites yielding long-chain fatty acids such as conjugated diene (CD)18:3, CD20:3, and CD20:4 (Banni et al., 2001, Banni, 2002). These CLA derivatives seem to replenish reduced levels of ARA that were previously observed in different tissues when CLA was fed (Banni et al., 1999). The resulting reduction of ARA availability might affect the production of eicosanoids (Banni, 2002), which were repeatedly shown to be decreased after CLA supplementation (Sugano et al., 1998; Li et al., 1999, Ringseis et al., 2006).

In addition to its potential influence on eicosanoid production, the effects of CLA might be mediated via activation of the transcription factor PPAR-α (Moya-Camarena et al., 1999). Previous studies indicate that the expression of multiple genes might be modulated by an enhanced CLA supply, including upregulation of uncoupling protein-2, by which energy expenditure might be increased (Ryder et al., 2001; Choi et al., 2004), and increased expression of adiponectin mRNA potentially alleviating hyperinsulinemia (Nagao et al., 2003). Furthermore, CLA can reduce body fat, inhibiting the differentiation of adipocytes and lipoprotein lipase activity of adipocytes (Park et al., 1997; Brodie et al., 1999). In rats, CLA supplementation led to lower serum insulin-like growth factor (IGF)-I levels, whereas CLA supplementation increased levels of IGF-binding proteins (IGFBP), which included mainly IGFBP-3, when combined with n-6 fatty acids and reduced levels of these binding proteins when combined with n-3 fatty acids (Li et al., 1999).

Thus, the competition with other PUFA might play a significant part in the exertion of CLA effects (Banni, 2002). However, not only CLA can affect the availability of EFA metabolites

but also an inhibition of the CLA metabolism by EFA might be possible. For instance, PUFA were previously shown to inhibit the activity of Δ-9 desaturase limiting CLA synthesis (Sessler et al., 1996; Nudda et al., 2008). Consequently, interactions between the fatty acids might affect both, EFA and CLA metabolism.

## 1.3 Essential fatty acids and conjugated linoleic acids in dairy nutrition

Pasture has traditionally been a major component in dairy nutrition. The composition of the fatty acids in pasture depends on multiple factors such as growth stage, species, fertilization, and season (Boufaied et al., 2003). Nevertheless, ALA has repeatedly been identified as most abundant fatty acid in pasture (Bauchart et al., 1984; Kay et al., 2005). However, in modern dairy nutrition, pasture is commonly replaced by total mixed rations, which often contain corn silage as main component. Compared to pasture, corn silage contains lower levels of ALA but higher levels of LA (Figure 1.3) (Ferlay et al., 2006), which leads to a higher n-6:n-3 ratio in the diet (Kay et al., 2005). Consequently, lower concentrations of ALA and higher concentrations of LA were observed in plasma and milk from cows receiving a corn silage-based TMR compared to pasture-fed cows (Kay et al., 2005).

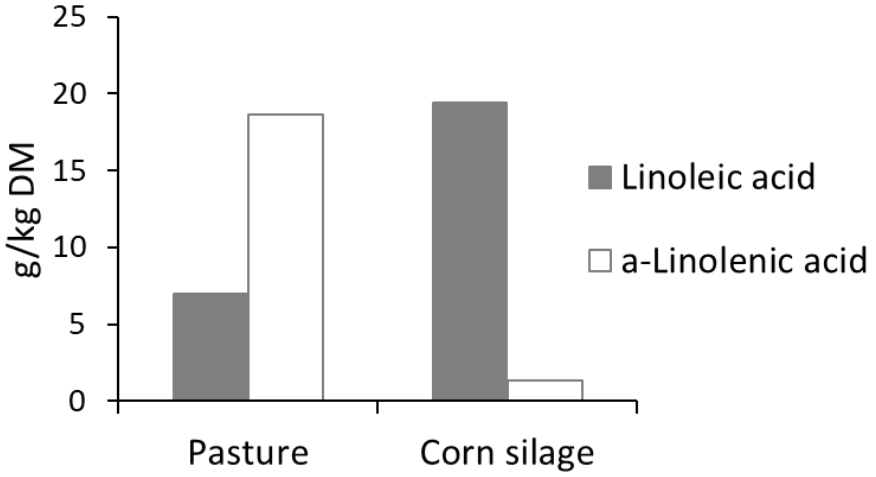


**Figure 1. 3: Content of linoleic acid and α-linolenic acid in pasture and corn silage. Adapted from Ferlay et al. (2006).**

Furthermore, feeding corn-silage based diets instead of pasture reduces CLA levels in milk fat as shown in previous studies. For instance, Kay et al. (2005) found 0.7% *cis*-9, *trans*-11 CLA in milk fatty acids from cows fed a corn silage-based total mixed ration (TMR) vs. 1.8% if cows were fed pasture. In a study from Jahreis et al. (1997), methyl esters comprised only 0.3% *cis*-9, *trans*-11 CLA in milk fat from cows receiving corn silage-based diets compared to 0.6% in milk fat from cows fed pasture during summer and grass silage or corn silage during other seasons. It is assumed, that higher *cis*-9, *trans*-11 CLA levels in milk from pasture fed cows originate from a higher ruminal vaccenic acid production but also an elevated Δ-9 desaturase activity might be responsible for this increase (Lock and Garnsworthy, 2003; Kay et al., 2005). In contrast, a concentrate-rich TMR with low fiber content might change the ruminal environment leading to an increased production of *trans*-10, *cis*-12 CLA and its intermediates (Griinari et al., 1998; Kay et al., 2005). Thus, the fatty acid supply from modern corn silage-dominated dairy rations might deviate considerably from the fatty acid supply provided by pasture. However, an altered supply with EFA and CLA might not be limited to the cow but could also affect the availability of fatty acids for its offspring.

## 1.4 Fatty acid supply of the calf

### *1.4.1 Placental fatty acid supply*

Long-chain PUFA play an important role in the development of the fetus, particularly the ALA derivative DHA is critical for the development of the nervous system (Koletzko et al., 2007; Innis, 2008). To meet its demand with these crucial fatty acids, the fetus depends on the fatty acid supply from the maternal circulation but in the bovine epitheliochorial placenta especially the transfer of PUFA is low (Crawford et al., 1981; Campbell et al., 1994; Moallem and Zachut, 2012). Thus, vast differences exist between the concentrations of EFA in maternal and fetal plasma. While LA accounts for up to 40% of the fatty acids in maternal plasma of

ruminants, the proportion of this fatty acid in fetal plasma fatty acids often ranges between 2 - 5 % (Noble et al., 1978a; Moallem and Zachut, 2012; Garcia et al., 2014a). It is assumed that the maternofetal fatty acid transfer is partly based on a concentration gradient across the placenta (Noble et al., 1978a; Moallem and Zachut, 2012). However, the fatty acid transfer to the fetus occurs mainly in the form of non-esterified fatty acids (NEFA) from the maternal plasma, which is the least abundant lipid fraction in the ruminant dam (Shand and Noble, 1981; Noble et al., 1982).

The transfer of EFA is further limited as ruminants retain these fatty acids efficiently by incorporating them mainly in phospholipids and cholesterol esters and assimilating only low proportions in NEFA and triglycerides (Noble et al., 1978a; Noble et al., 1978b). In addition to passive diffusion, fatty acids in the ruminant placenta might also be transferred via selective transportation (Salehi and Ambrose, 2017). In cotyledons of the ruminant placenta, genes for fatty acids transporters such as fatty acid transport proteins (FATPs), fatty acid translocase (FAT/CD36), and fatty acid-binding proteins (FABPs) are expressed, which were shown to promote fatty acid transfer in the human placenta and partly prefer EFA and their metabolites over non-EFA (Campbell et al., 1998; Dutta-Roy, 2000).

Another cause of the distinct differences between maternal and fetal plasma fatty acid compositions might be the considerable desaturation of EFA in the ruminant placenta, which possesses an active Δ-6 and Δ-9 desaturase system (Shand and Noble, 1981). Arachidonic acid from placental LA desaturation can subsequently serve as precursor of uterine prostaglandin F2α (PGF2α) (Mattos et al., 2004; Silvestre et al., 2011), but it can also be transferred to the fetus, making the placenta the main source of ARA for the fetus (Noble et al., 1982; Duvaux-Ponter et al., 2008). Nevertheless, previous studies repeatedly reported that neonatal ruminants show signs of EFA deficiency (Palmquist et al., 1977; Noble et al., 1982; Garcia et al., 2014a). Thus, a high triene:tertraene ratio and in particular a high occurrence of mead acid (20:3 *cis*-5, *cis*-8, *cis*-11, n-9) compared to ARA were previously described as indicators for biochemical

EFA deficiency as oleic acid can be desaturated and elongated to form mead acid when EFA availability is low (Holman, 1978; Palmquist, 2009).

Whether maternal LA supplementation can serve as a measure to increase the supply with LA and its derivatives for newborn ruminants was subject of several studies (Noble et al., 1978b; Elmes et al., 2004; Garcia et al., 2014a). Although maternal LA supplementation led primarily to an increased proportion of ARA in plasma, an increase of the LA proportion in the fetus is also possible if dams are supplemented with this fatty acid (Noble et al., 1978b; Elmes et al., 2004). In addition, it was shown that maternal LA supplementation can decrease the availability of long-chain n-3 fatty acids for the fetus, presumably due to a lower ALA desaturation in response to the increased competition with LA for desaturases (Garcia et al., 2014a).

Similar to LA levels, the proportions of ALA are low in the ruminant fetus (Noble et al., 1978a). Although studies addressing the impact of maternal ALA supplementation on the availability of n-3 fatty acids for the fetal calf are scarce, previous results indicate that the n-3 status of neonatal ruminants might be increased by maternal supplementation. For instance, Duvaux-Ponter et al. (2008) observed higher percentages of ALA and EPA in plasma fatty acids of goat kids, whose dams received ALA-rich extruded linseed compared to dams fed extruded rapeseed. In a study by Moallem and Zachut (2012) proportions of DHA were 1.9 times higher in calves, whose dams received DHA-rich fish oil during late gestation compared to the control group and maternal and calf plasma DHA levels correlated.

Thus, a transfer of n-3 fatty acids to the fetal ruminant seems possible although the extent to which maternal supplementation can increase the n-3 fatty acid status of the fetal calf remains unclear. In addition, CLA might be transferred to the fetus during gestation as previously indicated in humans and rats (Ringseis et al., 2004; Müller et al., 2007). As studies addressing this topic are scarce, little is known about the availability of CLA for the fetal calf and its modulation by the maternal fatty acid supply. Although Dänicke et al. (2012) demonstrated that

*cis*-9, *trans*-11 CLA is already present in erythrocyte lipids of newborn calves before first colostrum intake, maternal CLA supplementation during gestation only tended to increase the levels of *cis*-9, *trans*-11 in calves.

### *1.4.2 Fatty acid supply via colostrum and milk*

Unlike the considerably limited transfer of EFA to the fetal ruminant during gestation, the transfer of these fatty acids via colostrum and milk seems to be less tightly regulated (Garcia et al., 2014a). It was repeatedly observed in previous studies that signs of a low EFA status in newborn ruminants were reversed within the first days after birth when the animals consumed colostrum and milk (Noble et al., 1972; Noble et al., 1975; Garcia et al., 2014a). Especially colostrum might contribute to the neonate´s EFA supply as it contains higher amounts of PUFA and in particular higher amounts of ALA and its derivatives than mature or transition milk (Dänicke et al., 2012; Contarini et al., 2014; O'Callaghan et al., 2020). In contrast, the concentration of CLA increases postpartum and is significantly lower in colostrum than mature milk (Dänicke et al., 2012; Contarini et al., 2014). Although *cis*-9, *trans*-11 CLA is the major CLA isomer and LA and ALA are the predominant PUFA in milk fat, with only 5% the proportion of PUFA in milk fat is generally low (Savoini et al., 2016).

However, the levels of LA, ALA, and CLA in milk fat can be affected by fatty acid supplementation. Thus, an increase of ALA and CLA in milk fat can not only be achieved by the aforementioned feeding of pasture instead of corn-silage based rations (Kay et al., 2005), but also by the systematic supplementation of these fatty acids as shown in numerous studies (Pires and Grummer, 2008; Moallem, 2018). Due to variable ruminal biohydrogenation of the fatty acids in response to the supplementation technique, the extent to which the levels of the supplemented fatty acids can be increased by supplementation varies between different studies (Table 1.1).

**Table 1.1: Impact of fatty acid supplementation on milk fatty acid composition**

| Reference | Treatment | LA | ALA | EPA | DPA | CLA (*c*-9, *t*-11) | CLA (*t*-10, *c*-12) |
|---|---|---|---|---|---|---|---|
| | | % of total fatty acids in milk | | | | | |
| Petit et al., 2007 | Saturated fat | 2.4 | 0.73 | 0.04 | - | - | - |
| | Whole linseed | 3.0 | 1.09 | 0.11 | | | |
| Zachut et al., 2010 | No supplement | 4.60 | 0.25 | 0.02 | 0.04 | - | - |
| | Encapsulated flaxseed | 4.15 | 1.65 | 0.09 | 0.12 | | |
| Hötger et al., 2013 | Sunflower oil | 2.32 | 0.48 | - | - | 0.54 | 0.01 |
| | Lutrell (CLA) | 2.50 | 0.51 | | | 0.64 | 0.03 |
| Haubold et al., 2020 | Coconut oil | 2.49 | 0.22 | 0.03 | 0.06 | 0.39 | 0.01 |
| | Linseed+safflower oil | 3.98 | 4.86 | 0.09 | 0.15 | 0.33 | 0.01 |
| | Lutalin (CLA) | 4.03 | 0.38 | 0.02 | 0.06 | 1.87 | 0.79 |

For instance, a 1.5-fold increase in milk ALA proportions was reported by Petit et al. (2007) for cows fed whole flaxseed compared to cows receiving saturated fat, while Haubold et al. (2020) observed a 13-fold increase in cows that received abomasal infusions of linseed oil compared to the control group. Similarly, the extent to which the portion of the n-3 fatty acid derivatives EPA and DPA were increased by ALA supplementation varies between previous studies with reported increases such as 2.8- to 4.5- fold for EPA or 2.5- to 3- fold for DPA, whereas DHA is often not detected or very low in milk (Petit et al., 2007; Zachut et al., 2010, Haubold et al., 2020). For *cis*-9, *trans*-11 CLA in milk fat 1.2- and 4.8- fold increases and for *trans*-10, *cis*-12 CLA 3- and 79- fold increases were reported for cows fed rumen protected CLA or receiving abomasal infusions of CLA compared control groups, respectively (Hötger et al., 2013; Haubold et al., 2020).

The changes of the fatty acid composition in colostrum and milk might also affect the fatty acid status of young ruminants. Thus, feeding milk replacers with porcine lard, which provided additional LA and ALA, instead of coconut oil, led to increased proportions of these fatty acids as well as a total increase of n-3 and n-6 fatty acids in plasma of 30 d old calves (Garcia et al., 2014b). Similarly, adding linseed oil, fish oil and α-tocopherol to colostrum increased the levels

of ALA, EPA and DPA in plasma of calves during the first week after feeding (Opgenorth et al., 2020). Postpartum supplementation of ewes with fish oil led to higher levels of EPA, DPA, DHA, and *cis*-9, *trans*-11 CLA in milk fat that were reflected by higher levels of these fatty acids in intramuscular fat of lambs consuming this milk (Gallardo et al., 2014). An increase of *cis*-9, *trans*-11 CLA proportions could not be observed in colostrum from cows supplemented with CLA during gestation. Consequently, CLA concentrations in the erythrocyte lipids of their calves were not enhanced by suckling colostrum from CLA supplemented dams (Dänicke et al., 2012). However, in milk fat of ewes that were supplemented with extruded linseed during lactation, levels of ALA and *cis*-9, *trans*-11 CLA were increased and correlated with the levels of these fatty acids in the *longisssimus dorsi* muscle of their kids (Nudda et al., 2008).

### *1.4.3 Incorporation of essential fatty acids and conjugated linoleic acids*

Essential fatty acids supplied via the placenta or milk are widely retained by the young ruminant that is adapted to an efficient utilization of these fatty acids (Noble et al., 1972). Compared to stearic acid, the level of LA oxidation was 95% lower in sheep, and on starvation, the non-EFA palmitic, stearic, and oleic acid were preferentially mobilized in comparison to LA (Lindsay and Leat, 1977). Furthermore, the retention not only varies between different fatty acids but also between the lipid classes into which the fatty acids can be incorporated. Plasma lipids of the ruminant fetus as well as of 4-week old calves comprise mainly cholesterol esters (CE) and phospholipids (PL), which represent approximately 50 and 30% of the plasma lipids, respectively, whereas less than 10% of plasma lipids are triglycerides (TG) and NEFA (Noble et al., 1982; Jenkins et al., 1985).

Essential fatty acids are incorporated primarily into CE (Jenkins et al., 1985). Their synthesis is catalyzed by lecithin–cholesterol acyltransferase, which has a high affinity for EFA (Noble et al., 1973). In young ruminants, the proportion of LA in plasma CE increases strongly during the first weeks after birth reaching more than 50% whereas proportions of ALA range between

3 and 6% in four-week-old calves (Noble et al., 1975; Jenkins et al., 1985). Together with PL, CE are transported in low-density lipoproteins in the plasma, which facilitates the distribution of EFA throughout the body for the synthesis of membranes and messenger molecules (Drackley, 2005).

In addition to CE, considerable levels of EFA are also incorporated into PL, into which LA is incorporated to a greater degree than the saturated fatty acid stearic acid (Lindsay and Leat, 1977). Thus, percentages of LA in plasma PL of calves range between 10 and 20% during the first weeks after birth (Noble et al., 1975). In addition to the role of PL as membrane compartments, fatty acids that are released from PL by phospholipases can act as signaling molecules and precursors for eicosanoids (Innis, 2005). Lysophospholipids serve as fatty acid sources for various tissues such as the brain that takes up EFA derivatives in this form (Lagarde et al., 2001). The preferential incorporation of EFA into CE and PL allows an efficient EFA retention due to the low turnover of these lipid classes and their low incorporation into tissues that have a high consumption of non-EFA such as adipose or mammary tissue (Palmquist, 1976; Noble, 1979; Drackley, 2005).

Nevertheless, EFA are also found in plasma TG although the postnatal increase of EFA in this lipid class is distinctly lower compared to CE and PL (Noble et al., 1975). This low incorporation into TG does not apply for all PUFA, tough. Conjugated linoleic acids are incorporated mainly into neutral lipids, such as TG, and accumulate therefore particularly in neutral lipid-rich depot fat as previously observed in veal calves (Banni et al., 2001; Banni, 2002; Marounek et al., 2008). Fatty acids released from adipose tissue do not only serve as energy source, they can also be incorporated into PL and serve as precursors for lipid mediators (Contreras et al., 2012; Raphael and Sordillo, 2013). Plasma TG can be hydrolyzed by lipoprotein lipase to form NEFA that are taken up by various tissues such as adipose tissue, skeletal muscle, and liver (Drackley, 2005). Non-esterified fatty acids can subsequently be oxidized as energy source but they can also be incorporated into PL and provide fatty acids for

various tissues such as the brain (Jones et al., 1997; Drackley, 2005; Raphael and Sordillo, 2013). Thus, the lipid class into which the fatty acids are incorporated can determine the fatty acid´s subsequent utilization and the efficiency of their retention.

## 1.5 Energy metabolism of the calf

The fetal ruminant obtains major parts of its energy from maternal glucose and amino acids, whereas acetate, NEFA, and keto-acids only account for a small fraction of the energy supply (Bell et al., 2005). Significant quantities of maternal glucose are converted to lactate and smaller portions are metabolized to fructose in utero-placental tissues before they are transferred to the fetus and slowly metabolized (Meznarich et al., 1987; Bell et al., 2005). After birth, young ruminants obtain glucose from lactose in colostrum and milk but as the lactose supply does not meet the glucose requirements, neonates need to produce glucose endogenously, whereby glycogen serves as important but limited glucose source immediately after birth so that hypoglycemia is not uncommon in neonates (Mellor and Cockburn, 1986). In addition to glycogenolysis, young calves can synthesize glucose from precursors, such as lactate, amino acids, and glycerol via gluconeogenesis, which increases with age (Girard et al., 1992; Steinhoff-Wagner et al., 2011; Hammon et al., 2012).

Fat depots in the neonate are small and thus lipolysis is still low in the early postnatal phase, although the neonate is already able to mobilize fat to release NEFA for energetic purpose when the energy intake is low (Girard et al., 1992; Hadorn et al., 1997; Hammon et al., 2012). With the onset of milk intake, the fat supply to the newborn increases and relevant amounts of the long-chain fatty acids in milk are stored in adipose tissue (Drackley, 2005). Furthermore, mid- and long-chain fatty acids provided by milk are used as energy source by oxidation (Drackley, 2005; Hammon et al., 2012). While the energetic use of fatty acids by oxidation and ketogenesis in the fetal liver is still low, the capacity for fatty acid oxidation increases soon after birth (Girard et al., 1992; Odle et al., 1995; Hammon et al., 2012). Particularly when the supply with

carbohydrates or substrates for gluconeogenesis is depleted, ketone bodies such as β-hydroxybutyrate (BHB) are produced from mobilized fatty acids, although ketogenesis is still low in neonatal calves (Girard et al., 1992; Hammon et al., 2012; Raphael and Sordillo, 2013).

Already in early gestation, insulin and glucagon can be found in the pancreas of the fetus (D`Agostino et al., 1985; Bell et al., 2005). While insulin is known to increase glucose utilization in the fetus, the role and regulation of glucagon in the fetal metabolism is less clear (Hay Jr and Meznarich, 1986; Bell et al., 2005). In neonatal calves, plasma insulin levels are enhanced by the uptake of nutrients or glucose in particular (Hadorn et al., 1997; Hammon et al., 2012). The response of peripheral tissues to insulin in neonates is already comparable to older animals, whereas the inhibitory effect of insulin on gluconeogenesis in the liver is not fully developed yet (Farrag et al., 1997; Scheuer et al., 2006; Hammon et al., 2012). Plasma glucagon levels are modulated by feed intake in neonatal calves but they also depend on ontogenesis (Hammon and Blum, 1998; Steinhoff-Wagner et al., 2011; Hammon et al., 2012). In addition to the glucose regulating properties of insulin and glucagon, insulin was shown to promote protein anabolism, reduce amino acid oxidation, reduce lipolysis, and stimulate the synthesis of IGF-I (McGuire et al., 1995; Gingras et al., 2007; Pires and Grummer, 2008; Hammon et al., 2012).

During late gestation, IGF-I stimulates growth and organ development of the fetus (Breier et al., 2000; Gluckman and Pinal, 2003). As IGF-I is modulated by insulin (Gluckman et al., 1987), the glucose supply via the placenta is the most important regulator of IGF-I synthesis in the fetus that reacts to maternal starvation with reduced IGF-I concentrations in plasma (Oliver et al., 1993; Holt, 2002). The somatotropic axis with its constituents pituitary growth hormone (GH) and IGF-I is essential for postnatal growth (Breier and Gluckman, 1991). The mature somatotropic axis reacts to malnutrition with an increased production of GH and GH resistance regarding the IGF-I secretion due to a reduced GH receptor expression (Breier and Gluckman, 1991). In the neonate, plasma IGF-I levels are low and GH levels do not consistently respond

to nutrient intake, yet (Hammon et al., 2012). Nevertheless, IGF-I secretion can already be stimulated by an increased nutrient intake in the early postnatal phase (Hadorn et al., 1997; Hammon and Blum, 1997; Hammon et al., 2012; Frieten et al., 2018). In addition, the action of IGF-I is mediated by IGFBP to which the majority of IGF in the circulation and extracellular space are bound (Holt, 2002). During malnutrition, plasma levels of IGFBP-2 increase whereas IGFBP-3 levels decrease (Thissen et al., 1994; Jones and Clemmons, 1995). If it is bound to IGFBP-2, more IGF-I might be cleared from the circulation as IGFBP-2 is smaller than IGFBP-3 and could leave the capillaries more easily (Jones and Clemmons, 1995; Blum and Hammon, 2000). Subsequently, altered levels of IGF-I can affect cell proliferation and cell death, thereby modulating postnatal growth as previously indicated in dairy calves, in which increased average daily gain (ADG) coincided with increased plasma levels of IGF-I and IGFBP-3, but decreased plasma IGFBP-2 (Jones and Clemmons, 1995; Garcia et al., 2014b; Frieten et al., 2018).

The synthesis of the adipokines leptin and adiponectin is low in the ruminant fetus that only has small fat depots (Marple, 2003; Blum et al., 2005; Kesser et al., 2015). Compared to mature milk, colostrum contains higher concentrations of adiponectin and leptin, which seem to be absorbed and subsequently systemically available (Kesser et al., 2017; Liermann et al., 2020). Both hormones are associated with fat mass or accretion in early postnatal phase (Ehrhardt et al., 2003; Tsai et al., 2004). Thus, plasma levels of leptin can be increased by an enhanced nutrient supply in neonatal calves but the responsiveness of leptin to nutrition might be less pronounced in the first days of life (Block et al., 2003). The actions of leptin include increased energy expenditure, reduced lipogenesis, increased lipid oxidation, and decreased feed intake (Breier, 2006). Furthermore, adiponectin and leptin exert insulin-sensitizing effects (Havel, 2002; Kadowaki et al., 2006).

It cannot be excluded that maternal EFA and CLA supplementation might affect the energy metabolism of the calf by changing the nutrient supply via colostrum and milk. For instance, reduced milk protein contents were repeatedly observed in studies, in which cows were

supplemented with fat (Moallem, 2018), and supplementing ewes with ALA derivatives led to an increase of lactose levels (Nickles et al., 2019). In addition, CLA can reduce milk fat levels as reported in numerous studies (Bauman et al., 1999; Odens et al., 2007; Vogel et al., 2020). Thus, maternal EFA and CLA supplementation might affect the calf by changing the supply with nutrients from milk and by directly affecting the energy metabolism of the calf.

## 1.6 Impact of the fatty acid supply on the energy metabolism and development of calves

Previous studies indicate that a variable EFA supply can modulate aspects of the energy metabolism in calves. Thus, feeding calves milk replacer containing porcine lard, which provides additional LA and ALA, instead of coconut oil, increased plasma levels of glucose and decreased plasma urea levels in calves during the first 60 d of age (Garcia et al., 2014b; Garcia et al., 2016a). Accordingly, a higher EFA supply during fetal life might decrease the catabolism of sugars and amino acids as indicated by downregulation of genes coding proteins that enhance sugar and amino acid degradation in calves, whose dams received a LA-rich EFA supplement during gestation (Garcia et al., 2016b). In 3-month-old calves, concentrations of urea in serum decreased but also serum glucose concentrations decreased with increasing proportions of ALA-rich linseed oil in their starter (Hill et al., 2009). Furthermore, the addition of LA and ALA to milk replacer instead of coconut oil was shown to decrease BHB concentrations in plasma as well as the fat concentration in the liver of calves (Garcia et al., 2014b; Garcia et al., 2016b). It was assumed that these decreased fat concentrations in the liver were the result of an enhanced hepatic fatty acid oxidation, which was induced by EFA via the activation of PPARα (Garcia et al., 2016b). In addition, the uptake of circulating NEFA by peripheral tissues might be facilitated by ALA as previously indicated in non-lactating dairy cows (Mashek et al., 2005). Accordingly, plasma concentrations of metabolic hormones might be affected by a variable fatty acid supply. Previous studies indicate that levels of insulin and

IGF-I in plasma can be increased by an enhanced EFA supply as shown in calves receiving milk replacer with additional LA and ALA instead of coconut oil (Garcia et al., 2014b; Garcia et al., 2016a).

Furthermore, the aforementioned modulations of the metabolism by an enhanced EFA supply might coincide with an improved performance as repeatedly shown in previous studies. For instance, calves that received milk replacer enriched with LA and ALA had a higher ADG and an improved feed efficiency (Jenkins and Kramer, 1986; Garcia et al., 2014b; Garcia et al., 2016a). A gradual increase of ALA-rich linseed oil in the starter led to a linear increase of ADG and hip width change and tended to increase feed efficiency and starter intake of calves (Hill et al., 2009). It cannot be excluded that the development and functionality of certain organs might be modulated by an increased EFA supply. Thus, shorter villi and crypts were observed in piglets, whose dams received linseed oil instead of lard during lactation and gestation, and maternal supplementation with DHA increased glucose transport in the jejunum of piglets (Gabler et al., 2007; Boudry et al., 2009).

Little is known about the impact of an increased CLA supply on the metabolism and development of calves. Petzold et al. (2014) observed no impact of maternal CLA supplementation in the last three weeks of gestation on birth weight or serum metabolites of one-day-old calves. However, previous studies in other mammalian species indicate that modulations of the metabolism by CLA might be possible, including a reduction of adipose tissue weight and serum leptin levels, increased energy expenditure, and enhanced fat oxidation (West et al., 1998; Ohnuki et al., 2001; Masso-Welch et al., 2004).

## 1.7 Conclusive remarks and need for further research

Various studies indicate that EFA and CLA can modulate aspects of the metabolism and development in mammals. While it is known that modern corn silage-based rations change the cow´s supply with EFA and CLA compared to pasture, it is unclear whether an altered maternal

EFA and CLA status can also be transferred to the calf during gestation and via milk intake and whether it can modulate aspects of its metabolism and development.

To address these knowledge gaps, the present thesis aims to investigate the impact of maternal supplementation with EFA, CLA, and the combination of both during late gestation and early lactation on the fatty acid status and aspects of the metabolism and development of newborn calves. We hypothesized that these maternal treatments modulate the fatty acid composition in plasma of neonatal calves and affect the performance by altering the fatty acid supply or the nutrient supply via colostrum and transition milk. Furthermore, we hypothesized that maternal EFA and CLA supplementation modulates aspects of the energy metabolism, including endocrine factors related to neonatal glucose metabolism and growth, and that these changes are accompanied by effects on the development of the intestinal mucosa and further organs.

For this purpose, the calves´ plasma fatty acid compositions were analyzed directly after birth and after consumption of colostrum and transition milk from their dams during the five-day trial. Dams received corn silage-based diets and were supplemented with either coconut oil (CTRL), EFA in the form of linseed oil and safflower oil providing an n-6:n-3 ratio of 1:3, CLA in the form of Lutalin containing 27% of *cis*-9, *trans*-11 CLA and *trans*-10, *cis*-12 CLA, respectively, or a combination of both (EFA+CLA) from 63 days before calving until early lactation. Components of colostrum and transition milk were determined. Furthermore, the calves´ basal and postprandial metabolism was investigated by measurements of plasma metabolites and hormones, evaluation of energy expenditure and first-pass glucose uptake by tracer studies. Measurements of body and organ weight as well as intestinal morphometry were conducted to evaluate effects on the organ development.

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# CHAPTER 2

## Modulation of colostrum composition and fatty acid status in neonatal calves by maternal supplementation with essential fatty acids and conjugated linoleic acid starting in late lactation

**K. L. Uken,[1] C. T. Schäff,[1] L. Vogel,[1] M. Gnott,[1] D. Dannenberger,[2] S. Görs,[1] A. Tuchscherer,[3] A. Tröscher,[4] W. Liermann,[1] and H. M. Hammon[1*]**

[1]Institute of Nutritional Physiology "Oskar Kellner",

[2]Institute of Muscle Biology and Growth, and

[3]Institute of Genetics and Biometry, Leibniz Institute for Farm Animal Biology (FBN), 18196 Dummerstorf, Germany

[4]BASF SE, 68623 Lampertheim, Germany

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[*]Corresponding author: hammon@fbn-dummerstorf.de

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# 2. Modulation of colostrum composition and fatty acid status in neonatal calves by maternal supplementation with essential fatty acids and conjugated linoleic acid starting in late lactation


## Abstract

Sufficient maternal supply of essential fatty acids (EFA) to neonatal calves is critical for calf development. In the modern dairy cow, EFA supply has shifted from α-linolenic acid (ALA) to linoleic acid (LA) due to the replacement of pasture feeding by corn silage-based diets. As a consequence of reduced pasture feeding, conjugated linoleic acid (CLA) provision by rumen biohydrogenation was also reduced. The present study investigated the fatty acid (FA) status and performance of neonatal calves descended from dams receiving corn silage-based diets and random supplementation of either 76 g/d coconut oil (CTRL; n = 9), 78 g/d linseed oil and 4 g/d safflower oil (EFA; n-6/n-3 FA ratio = 1:3; n = 9), 38 g/d Lutalin (BASF SE, Ludwigshafen, Germany) providing 27% *cis*-9, *trans*-11 and *trans*-10, *cis*-12 CLA, respectively (CLA; n = 9), or a combination of EFA and CLA (EFA+CLA; n = 11) in the last 9 wk before parturition and following lactation. The experimental period comprised the first 5 d of life, during which calves received colostrum and transition milk from their own dam. The nutrient compositions of colostrum and transition milk were analyzed. Plasma samples were taken after birth and before first colostrum intake and on d 5 of life for FA analyses of the total plasma fat and lipid fractions. Maternal EFA and CLA supplementation partly affected colostrum and transition milk composition but did not change the body weights of calves. Most EFA in calves were found in the phospholipid (PL) and cholesterol ester (CE) fractions of the plasma fat. Maternal EFA supplementation increased the percentage of ALA in all lipid fractions of EFA and EFA+CLA compared with CTRL and CLA calves on d 1 and 5, and the increase was much greater on d 5 than on d 1. The LA concentration increased from d 1 to 5 in the plasma fat and

lipid fractions of all groups. The concentrations of docosapentaenoic acid, docosahexaenoic acid, and arachidonic acid in plasma fat were higher on d 1 than on d 5, and the percentage of n-3 metabolites was mainly increased in PL if dams received EFA. The percentage of *cis*-9, *trans*-11 CLA was higher in the plasma fat of EFA+CLA than CTRL calves after birth. By d 5, the percentages of both CLA isomers increased, leading to higher proportions in plasma fat of CLA and EFA+CLA than in CTRL and EFA calves. Elevated *cis*-9, *trans*-11 CLA enrichment was observed on d 5 in PL, CE, and triglycerides of CLA-treated calves, whereas *trans*-10, *cis*-12 CLA could not be detected in individual plasma fractions. These results suggest that an altered maternal EFA and CLA supply can reach the calf via the placenta and particularly via the intake of colostrum and transition milk, whereas the n-3 and n-6 FA metabolites partly indicated a greater transfer via the placenta. Furthermore, the nutrient supply via colostrum and transition milk might be partly modulated by an altered maternal EFA and CLA supply but without consequences on calf performance during the first 5 d of life.

**Key words:** essential fatty acids, conjugated linoleic acid, maternal fatty acid status, neonate

## 2.1 Introduction

Essential fatty acids (EFA) include the 2 fatty acids (FA) linoleic acid (LA; 18:2 *cis*-9, *cis*-12), an n-6 FA, and α-linolenic acid (ALA; 18:3 *cis*-9, *cis*-12, *cis*-15), which is classified as an n-3 FA (Burr and Burr, 1930; Neuringer et al., 1986). Both LA and ALA serve as precursors for PUFA of the n-3 and n-6 FA series, respectively, that are responsible for many essential functions, such as membrane fluidity, prostaglandin synthesis, and regulation of gene expression (Innis, 2005; Jump, 2008). Due to their critical role in cognitive development, the long-chain FA derived from LA and ALA are particularly important for the developing fetus (Koletzko et al., 2008). To obtain n-3 and n-6 FA, the fetus depends on the placental transfer of FA via maternal circulation (Innis, 2005). It was stated in previous studies that the transfer of EFA seems to be limited in the epitheliochorial placenta of ruminants, resulting in poor EFA

status of neonates (Noble et al., 1978a, 1982). Nevertheless, feeding LA to pregnant dams increased the proportion of this FA in the plasma of neonatal lambs (Noble et al., 1978b). However, EFA transfer by colostrum or milk seems to be of greater importance than placental transfer for neonatal EFA supply in calves (Garcia et al., 2014a, 2016). A significant increase in EFA in milk fat of the colostrum when cows are supplemented with EFA during late gestation was previously shown (Santschi et al., 2009; Garcia et al., 2014a). In addition, a recently published study indicated beneficial effects of n-3 FA supplementation with colostrum on the neonatal inflammatory response in calves (Opgenorth et al., 2020).

Furthermore, EFA can serve as a precursor for CLA, which is synthesized in the rumen (Bauman et al., 2000; Shingfield et al., 2010). Some CLA isomers have health-promoting effects in mammals (Nagao and Yanagita, 2005; Shokryazdan et al., 2017) but also reveal metabolic effects in dairy cows (Bauman et al., 2000; Hotger et al., 2013; Vogel et al., 2021). Studies in rats and pigs indicate placental and mammary transfers of CLA and that maternal CLA supply influences FA status, fetal development, and metabolism of the offspring (Park et al., 2005; Segovia et al., 2015). However, studies in cattle investigating the effect of maternal CLA supply on fetal and neonatal FA status and development are scarce. The maternal EFA and CLA status in modern dairy herds has changed due to the progressive replacement of pasture by corn silage-based diets (Barkema et al., 2015), which includes a shift from ALA to LA in the dairy ration, decrease of ALA in milk fat by about 300%, and reduced ruminant CLA biosynthesis and CLA in milk fat (about 250-300% less CLA in milk fat) because of the low EFA intake (Kelly et al., 1998; Kay et al., 2005; Couvreur et al., 2006; Lahlou et al., 2014). As the 2 EFA compete with each other for enzymes involved in the synthesis of their metabolites, changing the ratio of LA to ALA can affect the availability of their metabolites (Geiger et al., 1993; Calder, 2012).

Beyond the FA patterns, maternal supplementation with EFA may affect the nutrient composition of colostrum and milk (Moallem, 2018; Haubold et al., 2020) and the performance

of the offspring (Innis, 2005). Previous studies have shown that dietary supplementation of ALA alone or in combination with LA either to pregnant cows or directly to calves can improve the ADG and feed efficiency of calves (Hill et al., 2009; Garcia et al., 2014b). However, the effect of feeding colostrum and transition milk from dams with an elevated EFA and CLA supply on the development of neonatal calves remains unclear. In a recent study, we showed elevated concentrations of EFA, especially ALA and CLA, in the fat of colostrum and milk when cows were infused with linseed and safflower oil combined with or without CLA infusion during late gestation (Vogel et al., 2020). We hypothesized that changes in maternal EFA and CLA provision during late gestation and early lactation modify the FA status of calves, accordingly. In addition, maternal EFA and CLA supplementation may affect the performance of calves by influencing fetal FA supply and by changing the chemical composition and FA pattern in colostrum and transition milk. To address the FA status of the neonatal calves, FA patterns in calves were measured in several lipid fractions of the blood plasma before first colostrum feeding and on d 5 of life after colostrum and transition milk intake from their respective dam.

## 2.2 Materials and methods

The experimental procedures were conducted in strict accordance with the German Animal Welfare Act and were approved by the relevant Department for Animal Welfare Affairs of the state of Mecklenburg-West Pomerania, Germany (Landesamt fur Landwirtschaft, Lebensmittelsicherheit und Fischerei; LALLF M-V/ TSD/7221.3-1-052/15).

### *2.2.1 Animals, experimental design, and husbandry*

In the present study, the progeny from 37 German Holstein cows, which were part of an experiment addressing the effect of EFA and CLA during the transition phase, were investigated from December 2015 to September 2017. A detailed description of this experiment was previously published by Vogel et al. (2020). Briefly, dams were fed corn silage-based diets

providing a low content of EFA with especially low amounts of n-3 FA (1.4 g and 9.5 g of n-3 and n-6 FA per kg of DM, respectively) from the middle of second lactation (wk 22 antepartum) to third lactation. The treatments focused on the supply of FA that provide EFA (mainly ALA), CLA, or the combination of both. Such a treatment model refers to the supply of EFA and related rumen and tissue CLA production in dairy cows receiving fresh grass or on pasture (Kelly et al., 1998; Ferlay et al., 2006; Lahlou et al., 2014) and was described in detail by Vogel et al. (2020). Dams were randomly assigned according to milk yield and BW to 1 of 4 treatments: control group (CTRL) receiving 76 g/d coconut oil (Bio-Kokosöl #665, Kräuterhaus Sanct Bernhard KG, Bad Ditzenbach, Germany); EFA in the form of 78 g/d linseed oil (DERBY Leinöl #4026921003087, DERBY Spezialfutter GmbH, Münster, Germany) and 4 g/d safflower oil (GEFRO Distelöl, GEFRO Reformversand Frommlet KG, Memmingen, Germany; 38 g/d Lutalin (CLA; *cis*-9, *trans*-11, *trans*-10, *cis*-12 CLA, 10 g/d each; BASF SE, Ludwigshafen, Germany); and a combination of EFA and CLA supplementation (EFA+CLA; 78 g/d linseed oil + 4 g/d safflower oil + 38 g/d Lutalin). As previously reported by Vogel et al. (2020), the CTRL supplement provided less than 1.4 g/d EFA. The EFA supplement provided 39.9 g/d ALA and 14.9 g/d LA. Treatments for CTRL and EFA were iso-energetic. Doses for the supplied EFA (linseed and safflower oil in a ratio of 19.5:1; providing an n-6/n-3 FA ratio of 1:3 in the supplement mixture) and CLA were recently evaluated in a companion dose-response study in mid-lactating dairy cows (Haubold et al., 2020). Vitamin E was added to the CTRL and CLA supplements to compensate for naturally occurring vitamin E in linseed oil, which was applied in the EFA and EFA+CLA groups. The FA were supplemented to the cows from d 63 before calving until early lactation and were infused directly into the abomasum to avoid ruminal biohydrogenation (Vogel et al., 2020). The amount of supplements were halved during the dry period starting at 6 wk antepartum. For technical reasons, the study was subdivided into 5 consecutive blocks with 7 to 8 calves born per block. In total, 38 calves of the CTRL (5 male, 4 female), EFA (4 male, 5 female), CLA (1 male, 8 female), and EFA+CLA

(4 male, 7 female; 9 single and one pair of twins: one male, one female) groups were investigated from birth until d 5 of life. Originally 40 cows were fitted with rumen fistula and tube connection to the abomasum in the study (Vogel et al., 2020). Two cows calved prematurely and one calf died during the birth process. Therefore, the calves of 37 cows were included in this study.

Calves were separated from their dam directly after birth and were housed in a climate-controlled room at 19°C in single boxes with straw bedding and free access to water. Body weights were recorded before the first feeding after birth and at the end of the experiment on d 5 at 2 h after feeding. During the experiment, calves were fed colostrum (defined as milk of first 24 h after calving) and transition milk (defined as milk from d 2 to 5 after calving) from their own dam. If the colostrum quantity of a dam was insufficient, the required amount was achieved by combination with the colostrum from another cow of the same treatment group to ensure that the FA supply was consistent within the treatment group. This happened 4 times (in CTRL and EFA groups). The first meal was fed on average 2.5 ± 1.7 h after birth. Calves were fed colostrum from the first milking in amounts of 10% of BW during the first 24 h after birth divided into 2 meals. Colostrum from the second milking after calving was only fed if the amount of first colostrum was not sufficient for the second meal. On d 2 (after 24 h of birth and before beginning of d 3 of life), calves were fed transition milk from the third milking after calving; feed allowance was 6% of BW on d 2 to ensure that all calves received the same amount of transition milk before d 3 of life, irrespective of whether calves were born in the morning or afternoon the day before. From d 3 onward, calves were fed twice daily transition milk of the fifth, seventh, and ninth milkings after calving at 12% of BW/d divided into 2 meals in the morning and evening. The exact nutrient intakes of calves are presented in Table 2.1. Calves were fed by nipple bottle, and those refusing to drink were tube fed to ensure similar milk intake between individuals. Individual colostrum and milk samples from daily morning and afternoon milkings were taken and stored at -20°C until chemical composition analysis.

### *2.2.2 Milk analyses*

Chemical composition analyses were performed for colostrum and transition milk of all milkings (milkings 1-9 from d 1 to 5 after calving) of the study. Thawed samples were homogenized by warming (41°C) and pivoting before further analyses. Determinations of DM, protein, lactose, and fat were conducted according to the protocol of Görs et al. (2009). For determination of DM, triplicates of 8 µL of milk were dried in tin capsules (5 h, 55°C). The mean coefficient of variation for DM in triplicate was 0.6%. Dried samples were subsequently used for nitrogen determination with a mean coefficient of variation of 2.0% for triplicate nitrogen measurements by an elemental analyzer (EA 1108, Fisons Instr., Rodano Milan, Italy) coupled with a mass spectrometer (delta S interfaced to ConFlo II: Finnigan MAT, Bremen, Germany). Nitrogen was converted into CP by factor 6.38. Fat content was measured according to the Röse-Gottlieb-procedure, which was modified by Görs et al. (2009). A volume of 500 µL of milk diluted in 500 µL of ultra-pure water was used for analyses; the mixture was subjected to shaking for 60 s for separation of the ether soluble components in each extraction step. To verify the accuracy of single measurements, the same sample was measured 12 times in each block. The mean coefficient of variation of these repeated measurements was 2.7%.

For determination of lactose, 250 µL of milk was diluted with 1 mL of ultra-pure water. Diluted milk was centrifuged (50,000 × g for 20 min at 4°C), and the obtained clear solution was pipetted into a new tube, centrifuged again under the same conditions, and stored at -20°C until further analyses. The subsequent analysis of the lactose concentration in the clear solution was conducted as published by Görs et al. (2009) but with an increased sonication time of 5 min and a sample volume of 10 µL of 50-fold diluted solution measured by HPLC. The mean coefficient of variation for lactose measurements was 2.4%. The concentrations of the lactose and fat in milk on a g/kg basis were computed from concentrations on a g/L basis by correcting for the density of milk from the respective milking according to data from Madsen et al. (2004). The energy content in milk was estimated as follows: 17.0, 24.2, and 36.6 MJ of gross energy

per kg of lactose, protein, and fat, respectively; ME = 0.97 × 0.96 × gross energy (Kühne et al., 2000; NRC, 2001).

### *2.2.3 Analyses of plasma fatty acids*

Blood samples were taken directly after birth and before first colostrum intake by venipuncture from the jugular vein using evacuated tubes (1.2-2 mg of $K_3$EDTA/mL, Vacuette, Greiner Bio-One International GmbH, Kremsmünster, Austria). On d 5 of life, blood was sampled in S-Monovette tubes (1.6 mg of $K_3$EDTA/ mL, Sarstedt AG & Co., Nümbrecht, Germany) using a catheter (Cavafix Certo with Splittocan, B. Braun Melsungen AG, Melsungen, Germany) inserted into the jugular vein the day before. Blood samples were placed on ice before centrifugation at 2,700 × g and 4°C for 20 min. The obtained plasma samples were stored at -20°C until FA analyses.

Extraction of plasma lipids was conducted as described by Dannenberger et al. (2017). Thin-layer chromatography was used to separate triglycerides (TG), phospholipids (PL), cholesterol esters (CE), and free FA (FFA) in the extracted total plasma lipids as previously described (Dannenberger et al., 2017). After lipid class separation in a chromatography glass chamber, the thin-layer chromatography plates were dipped into a 0.03% 2.7-dichlorofluorescein solution (Chromatogram Immersion Device III, CAMAG, Muttenz, Switzerland) for visualization of the lipid fractions under UV light. The lipid fractions were scraped off the plate and rinsed with chloroform/methanol (2:1, vol/vol). The obtained extracts were filtered and reduced to dryness under a gentle nitrogen stream for subsequent transesterification and gas chromatography measurements.

For analyses of FA in total plasma lipids and lipid fractions, a capillary gas chromatograph with a CP-Sil 88 CB column (100 m × 0.25 mm, Agilent, Santa Clara, CA) was used, which was installed in a PerkinElmer gas chromatograph CLARUS 680 (PerkinElmer Instruments, Shelton, CT) with a flame ionization detector and split injection. Details of the chromatography

conditions were previously published by Dannenberger et al. (2012). Fatty acids were quantified using C19:0 as an internal standard. A reference standard mixture (Sigma FAME, Sigma-Aldrich, Deisenhofen, Germany) and methyl esters of C18:1 *cis*-11, C22:5n-3, C18:2 *cis*- 9, *trans*-11 (Matreya LLC, State College, PA), C22:4n-6 (Sigma-Aldrich), and C18:4n-3 (Larodan, Limhamn, Sweden) were used for calibration. The 5-point calibration was verified after analysis of 5 samples, and the results ranged between 16 and 415 µg/mL.

Fatty acid percentages below the detection limit of 0.01% were defined as 10% of the detection limit for statistical analyses. The percentage of *trans*-10, *cis*-12 CLA in lipid fractions was below the detection limit. One sample (EFA group, d 5) was excluded from statistical analysis of the FA composition in lipid fractions due to technical reasons. To estimate the activity of Δ5, Δ 6, and Δ 9 desaturase, the desaturase indices were calculated as the ratio [product]: [precursor + product] (Kelsey et al., 2003; Nudda et al., 2008). To estimate the relationship between calf and maternal FA, data on the FA composition in maternal plasma and colostrum from d 1 were obtained from Vogel et al. (2020) and Gnott et al. (2020).

### *2.2.4 Statistical analyses*

Statistical analyses were performed with SAS for Windows (version 9.4, SAS Institute Inc., Cary, NC) using the MIXED procedure. Data for nutrient intake, plasma FA composition, and BW were analyzed using the MIXED procedure by repeated-measures ANOVA containing EFA (level: yes, no), CLA (level: yes, no), time (levels: day relative to calving), block (levels: 1 to 5), sex, and their respective interactions (EFA × CLA; EFA × time; CLA × time; EFA × CLA × time) as fixed effects. For analyses of milk composition, the same model was used with the fixed effects EFA (level: yes, no), CLA (level: yes, no), milking number (levels: milking 1 to 9 after calving), block (levels: 1 to 5), and their respective interactions. The duration of maternal FA supplementation and gestation length were included as covariates. The REPEATED statement was utilized to consider measurements from the same calf or from milk

from the same dam applying an unstructured covariance matrix for analyses of FA composition and BW. Milk composition was analyzed using a compound-symmetry covariance structure. Pairwise differences in least squares means (LSM) were analyzed by the Tukey-Kramer test. Partitioned analyses of the LSM for interactions were conducted with the SLICE statement of the MIXED procedure. The results are presented as LSM ± SE unless otherwise stated. Effects were considered significant if $P < 0.05$.

To analyze the relationship between FA percentages in maternal and calf plasma as well as colostrum and calf plasma, Spearman´s rank-order correlations were applied using the CORR procedure of SAS.

## 2.3 Results

### *2.3.1 Milk composition, calf performance, and gestation length*

The DM content in the first milking after calving on d 1 was lower ($P < 0.01$) for dams that received CLA than for dams in the CTRL group (Figure 2.1A). The DM content in the second milking on d 1 was higher ($P < 0.05$) in dams receiving CLA than in dams in the EFA+CLA treatment. The protein concentration in the first milking on d 1 was lower ($P < 0.05$) in milk from dams supplemented with CLA than in that from dams that did not receive the CLA supplement (Figure 2.1B). The DM content and protein concentration in colostrum decreased ($P < 0.01$) from d 1 to 5 in all groups and were similar among groups from d 2 on. The fat concentration in milk variably changed with time ($P < 0.001$) during the first 5 DIM, showing the lowest concentration in the third milking and the highest concentration in the seventh milking (Figure 2.1C). The fat concentration was lower ($P < 0.05$) on d 1, 2 (fourth milking), and 3 (fifth milking) in milk of dams receiving EFA than in milk from those receiving no EFA treatment. In the first milking after calving, the fat concentration was higher ($P < 0.05$) in CTRL than in both EFA-treated groups. On d 2 (fourth milking), the fat concentration was higher ($P < 0.01$) in CLA than in EFA+CLA. The lactose concentration increased ($P < 0.001$) from d 1

to 5 in all groups (Figure 2.1D). The lactose concentration was higher ($P < 0.05$) on d 1 (2nd milking), d 3 (fifth milking), and d 4 (8th milking) in the milk of dams treated with EFA than in that from dams receiving no EFA treatment. In addition, the lactose concentration was higher ($P < 0.05$) on d 1 (2nd milking) in EFA+CLA than in CLA cows and was higher ($P < 0.05$) on d 4 (8th milking) in dams treated with EFA than in CTRL cows.

The protein and lactose intake did not differ among calves during the first 5 d of life, but fat and energy intake on d 1 and 3 were lower ($P < 0.05$) in EFA- than in non-EFA-treated calves (Table 2.1). The fat intake was higher ($P < 0.05$) on d 1 in CTRL than in EFA and was higher ($P < 0.05$) on d 3 in CLA than in EFA+CLA. Furthermore, energy intake on d 3 was higher ($P < 0.05$) in CLA than EFA+CLA. Despite differences in energy intake, the BW on d 5 as well as the birth weight were similar among groups (LSM ± SE were 43.6 ± 2.0 kg for birth weight and 45.7 ± 2.1 kg for BW on d 5). Accordingly, no difference was observed for ADG (LSM ± SE was 0.51 ± 0.05 kg/d). The weights of female and male calves were similar (data not shown).

Gestation time tended to increase if dams received EFA compared with that in dams without EFA supplementation ($P = 0.07$), with 278 ± 2 d and 277 ± 2 d (SEM ± SE) in EFA and EFA+CLA and 274 ± 2 and 276 ± 2 d in CTRL and CLA cows, respectively. Consequently, the supplementation period tended to be longer for dams receiving the EFA supplement than in those without EFA supplementation ($P = 0.08$), with supplementation times of 63.8 ± 2.8 and 63.2 ± 2.3 d (SEM ± SE) for EFA and EFA+CLA and 58.9 ± 2.8 and 61.0 ± 2.8 d for CTRL and CLA cows, respectively.

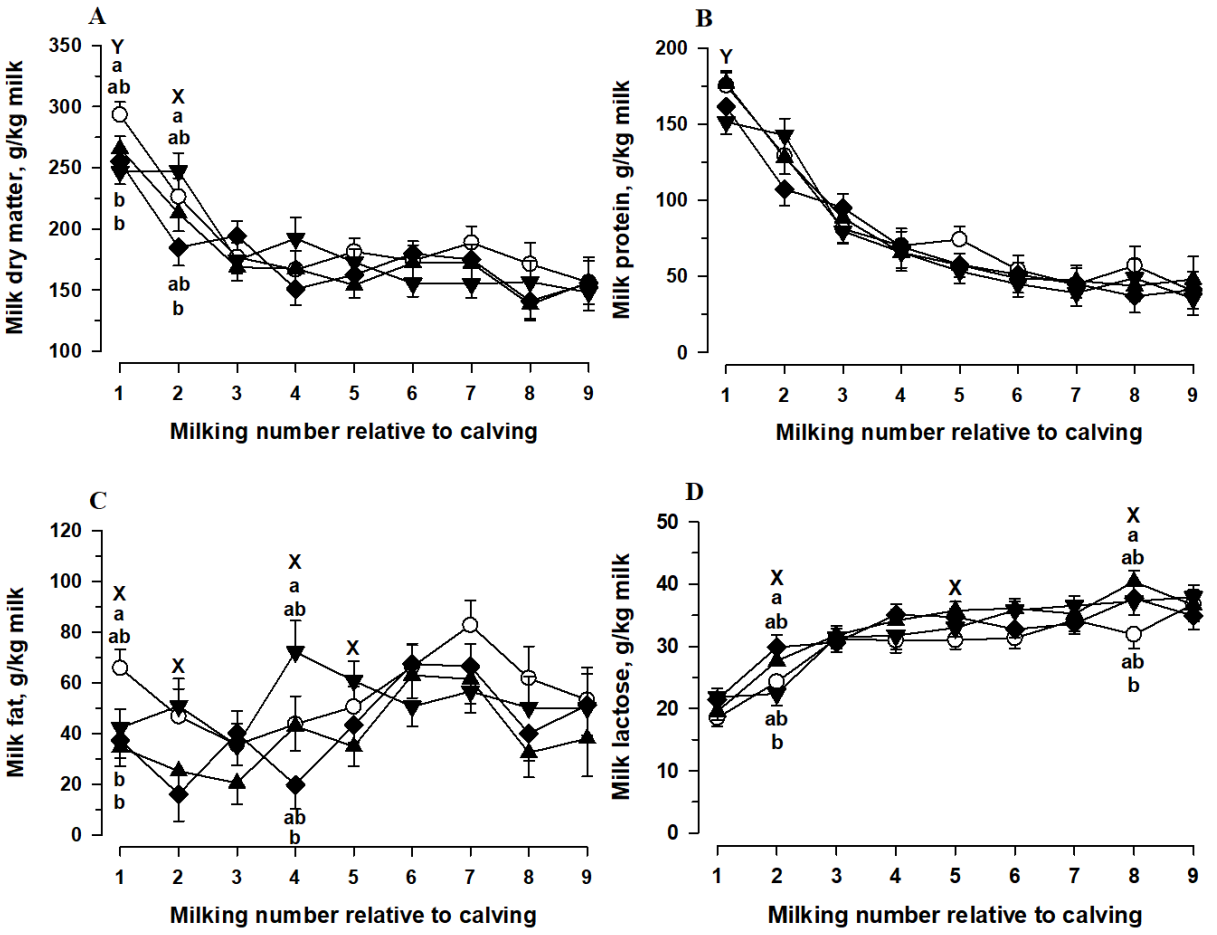


**Figure 2.1: Effects of supplementation with coconut oil (○ CTRL; n = 9), linseed and safflower oil (▲ EFA; n = 9), Lutalin (BASF SE, Ludwigshafen, Germany; ▼ CLA; *cis*-9, *trans*-11 and *trans*-10, *cis*-12; n = 9), or a combination of EFA and CLA (♦ EFA+CLA; n = 11) on the content of DM (A), protein (B), fat (C), and lactose (D) in fresh matter of milk from the first 9 milkings postpartum. Data are presented as LSM ± SE; different letters (a, b) indicate significant differences between groups; X indicates significant differences between EFA- and non-EFA-treated animals; Y indicates significant differences between CLA- and non-CLA-treated animals. The effect of EFA × time interaction on the milk lactose content and the effect of EFA on the milk fat content was significant ($P < 0.05$).**

**Table 2.1: Intake of milk protein, fat, and lactose during the first 5 d of life in calves, whose dams were supplemented with coconut oil (CTRL; n = 9), linseed and safflower oil (EFA; n = 9), Lutalin (BASF SE, Ludwigshafen, Germany; CLA; *cis*-9, *trans*-11 and *trans*-10, *cis*-12; n = 9), or a combination of the EFA and CLA (EFA+CLA; n = 11)[1]**

| | | Supplementation | | | | *P*-value | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Item | Time[2] | CTRL | EFA | CLA | EFA+CLA | EFA | CLA | EFA × CLA | Time | EFA × time | CLA × time |
| Protein | 1 | 16.73 ± 0.87 | 15.67 ± 0.85 | 16.15 ± 0.85 | 15.58 ± 0.77 | | | | | | |
| (g/kg BW) | 2 | 6.13 ± 0.87 | 5.52 ± 0.85 | 5.75 ± 0.85 | 6.12 ± 0.77 | | | | | | |
| | 3 | 9.27 ± 0.87 | 7.68 ± 0.85 | 7.57 ± 0.85 | 7.32 ± 0.77 | 0.40 | 0.47 | 0.52 | <0.001 | 0.79 | 0.73 |
| | 4 | 6.01 ± 0.96 | 5.77 ± 0.85 | 5.68 ± 0.89 | 5.72 ± 0.83 | | | | | | |
| | 5 | 5.45 ± 0.92 | 4.86 ± 0.85 | 4.86 ± 0.85 | 4.61 ± 0.77 | | | | | | |
| Fat | 1 | 6.11 ± 0.81[a] | 3.08 ± 0.80[b] | 4.38 ± 0.79[ab] | 3.31 ± 0.72[ab] | | | | | | |
| (g/kg BW) | 2 | 2.40 ± 0.81 | 1.33 ± 0.80 | 2.84 ± 0.79 | 1.74 ± 0.72 | | | | | | |
| | 3 | 5.82 ± 0.81[ab] | 5.10 ± 0.80[ab] | 7.76 ± 0.79[a] | 4.20 ± 0.72[b] | 0.02 | 0.61 | 0.41 | <0.001 | 0.14 | 0.51 |
| | 4 | 8.38 ± 0.90 | 7.20 ± 0.80 | 6.42 ± 0.83 | 7.64 ± 0.79 | | | | | | |
| | 5 | 7.33 ± 0.86 | 5.18 ± 0.80 | 5.51 ± 0.79 | 5.81 ± 0.72 | | | | | | |
| Lactose | 1 | 2.01 ± 0.24 | 2.35 ± 0.24 | 2.45 ± 0.23 | 2.66 ± 0.21 | | | | | | |
| (g/kg BW) | 2 | 1.89 ± 0.24 | 2.00 ± 0.24 | 1.92 ± 0.23 | 1.91 ± 0.21 | | | | | | |
| | 3 | 3.72 ± 0.24 | 4.31 ± 0.24 | 4.32 ± 0.23 | 4.24 ± 0.21 | 0.52 | 0.58 | 0.37 | <0.001 | 0.61 | 0.17 |
| | 4 | 4.03 ± 0.26 | 4.25 ± 0.24 | 4.16 ± 0.24 | 4.00 ± 0.23 | | | | | | |
| | 5 | 4.07 ± 0.25 | 4.07 ± 0.24 | 3.99 ± 0.23 | 3.86 ± 0.21 | | | | | | |

**Table 2.1: Continuation**

| Item | Time[2] | Supplementation | | | | *P*-value | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | CTRL | EFA | CLA | EFA+CLA | EFA | CLA | EFA × CLA | Time | EFA × time | CLA × time |
| Energy | 1 | 0.62 ± 0.04 | 0.50 ± 0.03 | 0.55 ± 0.03 | 0.51 ± 0.03 | | | | | | |
| (MJ ME/kg BW) | 2 | 0.25 ± 0.04 | 0.20 ± 0.03 | 0.26 ± 0.03 | 0.23 ± 0.03 | | | | | | |
| | 3 | 0.47 ± 0.04[ab] | 0.42 ± 0.03[ab] | 0.50 ± 0.03[a] | 0.38 ± 0.03[b] | 0.02 | 0.46 | 0.36 | <0.001 | 0.16 | 0.76 |
| | 4 | 0.49 ± 0.04 | 0.44 ± 0.03 | 0.41 ± 0.04 | 0.45 ± 0.03 | | | | | | |
| | 5 | 0.44 ± 0.04 | 0.35 ± 0.03 | 0.36 ± 0.03 | 0.36 ± 0.03 | | | | | | |

[a,b]Least squares means within a row with different lowercase letters differ between treatments ($P < 0.05$).

[1]Values are presented as LSM ± SE.

[2]Day of life.

### *2.3.2 Fatty acid composition of total plasma fat*

The plasma lipid content ranged between 0.06% and 0.08% ± 0.01% at birth and was increased by d 5 of life ($P < 0.001$), reaching 0.17% to 0.18% ± 0.01% (Supplemental Table S2.1). Despite similar plasma lipid contents between the groups, the proportion of various FA in the plasma was affected by the maternal treatment (Figure 2.2; Supplemental Table S2.1).

Concentrations of all measured FA in plasma fat and in blood plasma are presented in Supplemental Tables S2.1 and S2.2, respectively. The concentration of ALA in plasma fat was higher ($P < 0.01$) immediately after birth and before first milk intake in calves of both EFA groups than in CLA and CTRL calves (Figure 2.2A). Plasma ALA increased ($P < 0.01$) in all calves from d 1 to 5, but the marked increase in ALA in EFA and EFA+CLA calves resulted in a much higher ALA concentration ($P < 0.001$) in calves from both EFA groups than in CTRL and CLA calves on d 5 of life. The concentration of eicosapentaenoic acid (EPA) in plasma fat increased ($P < 0.01$) only in non-EFA-supplemented calves, and the concentrations of docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA) decreased ($P < 0.001$) in all groups from d 1 to 5 of life (Figure 2.2B-D). Concentrations of EPA and DHA as well as the sum of n-3 FA (Supplemental Table S2.1) were higher in plasma fat of EFA and EFA+CLA calves than in CTRL and CLA calves on d 1 and 5 ($P < 0.05$); for DPA, a higher concentration in individual EFA groups was only determined on d 5 ($P < 0.001$), but there was also an EFA effect with higher DPA concentrations in EFA- than non-EFA-treated calves on d 1. Moreover, the concentrations of ALA, EPA, DPA, and DHA in calf plasma fat at birth were positively correlated with the concentration of the respective FA in maternal plasma fat (Supplemental Table S2.3), with correlation coefficients ranging from 0.56 to 0.77 (Table 2.2). The proportions of ALA, EPA, and DPA in calf plasma fat on d 5 correlated with the concentration of these FA in the fat fraction of colostrum (Table 2.2).

**Table 2.2: Relationship between the fatty acid composition in maternal and calf plasma fat on d 1 and colostrum and calf plasma on d 5 of life**

| | Correlation | | | |
|---|---|---|---|---|
| | Dam – Calf[2] (n = 38) | | Colostrum – Calf[3] (n = 38) | |
| Fatty acid[1] | r | *P*-value | r | *P*-value |
| ALA | 0.74 | <0.001 | 0.85 | <0.001 |
| EPA | 0.77 | <0.001 | 0.79 | <0.001 |
| DPA | 0.56 | <0.001 | 0.75 | <0.001 |
| DHA | 0.65 | <0.001 | -[4] | |
| LA | 0.12 | 0.49 | 0.36 | 0.03 |
| ARA | 0.50 | <0.01 | 0.48 | <0.01 |
| c-9, t-11 CLA | 0.55 | <0.001 | 0.37 | 0.02 |
| t-10, c-12 CLA | 0.04[5] | 0.82 | 0.40 | 0.01 |

[1]ALA = α-linolenic acid; EPA = eicosapentaenoic acid; DPA = docosapentaenoic acid; DHA = dosahexaenoic acid; LA = linoleic acid; ARA = arachidonic acid.

[2]Relationship between fatty acids in maternal plasma fat and fatty acids in plasma fat of calves at birth and before first colostrum intake. Data for fatty acids in the plasma fat of cows are shown in Supplemental Table S2.3 and were first published by Gnott et al. (2020).

[3]Relationship between fatty acids in milk fat of first colostrum after calving and fatty acids in plasma fat of calves on d 5. Data for fatty acids in colostrum are shown in Supplemental Table S2.3 and were first published by Vogel et al. (2020).

[4] Not detected in colostrum.

[5]n = 37.

The concentrations of LA and total n-6 FA in plasma fat were similar among groups, but arachidonic acid (ARA) was lower ($P < 0.05$) in calves from EFA than non-EFA-treated dams on d 1 and 5 (Figure 2.2E, 2.2F; Supplemental Table S2.1). The concentration of LA increased ($P < 0.001$), but the concentration of ARA decreased ($P < 0.001$) from d 1 to 5 in all groups. On d 5, the concentration of ARA in plasma fat was higher ($P < 0.05$) in CTRL than in EFA and EFA+CLA. There was no relationship between the percentage of LA in plasma fat of dams

and calves on d 1 (Table 2.2). Nevertheless, the proportions of ARA in the plasma fat of newborn calves were related to those measured in their dams (Table 2.2). In addition, the concentrations of LA and ARA in milk fat were positively related to FA concentrations in calf plasma fat on d 5 (Table 2.2).

Prepartum supplementation of cows with CLA affected the *cis*-9, *trans*-11 CLA in the plasma fat of newborn unsuckled calves on d 1 ($P < 0.001$; Figure 2.2G). The concentration was higher ($P < 0.05$) in EFA+CLA than in CTRL and EFA and was higher ($P < 0.05$) in CLA than EFA. The concentration of *cis*-9, *trans*-11 CLA increased ($P < 0.001$) in all groups from d 1 to 5, was higher ($P < 0.001$) on d 5 in calves from CLA than non-CLA-treated dams, and was lower ($P < 0.05$) in calves from EFA- than non-EFA-treated dams. The concentration of *trans*-10, *cis*-12 CLA in plasma fat was similar among groups at birth, increased ($P < 0.001$) in calves from cows supplemented with CLA, but decreased ($P < 0.05$) in calves from non-CLA-supplemented dams, and was higher ($P < 0.001$) on d 5 in calves from CLA-treated dams than non-CLA-treated dams (Figure 2.2H). The concentration of *cis*-9, *trans*-11 CLA in calf plasma fat on d 1 was positively related to the concentration in maternal plasma fat (Table 2.2). Furthermore, positive correlations for both CLA isomers existed between colostrum fat and calf plasma fat on d 5 (Table 2.2).

On d 1, the Δ 5 desaturase index was higher in CTRL and CLA than in EFA and in EFA+CLA ($P < 0.01$; Supplemental Table S2.1). However, in EFA and EFA+CLA, the desaturase index increased ($P < 0.001$) from d 1 to 5, resulting in similar Δ 5 desaturase indices between groups on d 5. In contrast, the Δ 6 desaturase index was similar between groups on d 1, decreased ($P < 0.001$) from d 1 to 5 in all groups and was higher ($P < 0.01$) in CTRL than in EFA+CLA on d 5. The Δ 9 desaturase index was similar between groups on d 1 but decreased ($P < 0.01$) only in EFA and EFA+CLA from d 1 to 5. On d 5, the Δ 9 desaturase index was highest ($P < 0.01$) in CTRL and higher ($P < 0.05$) in CLA than in EFA+CLA.

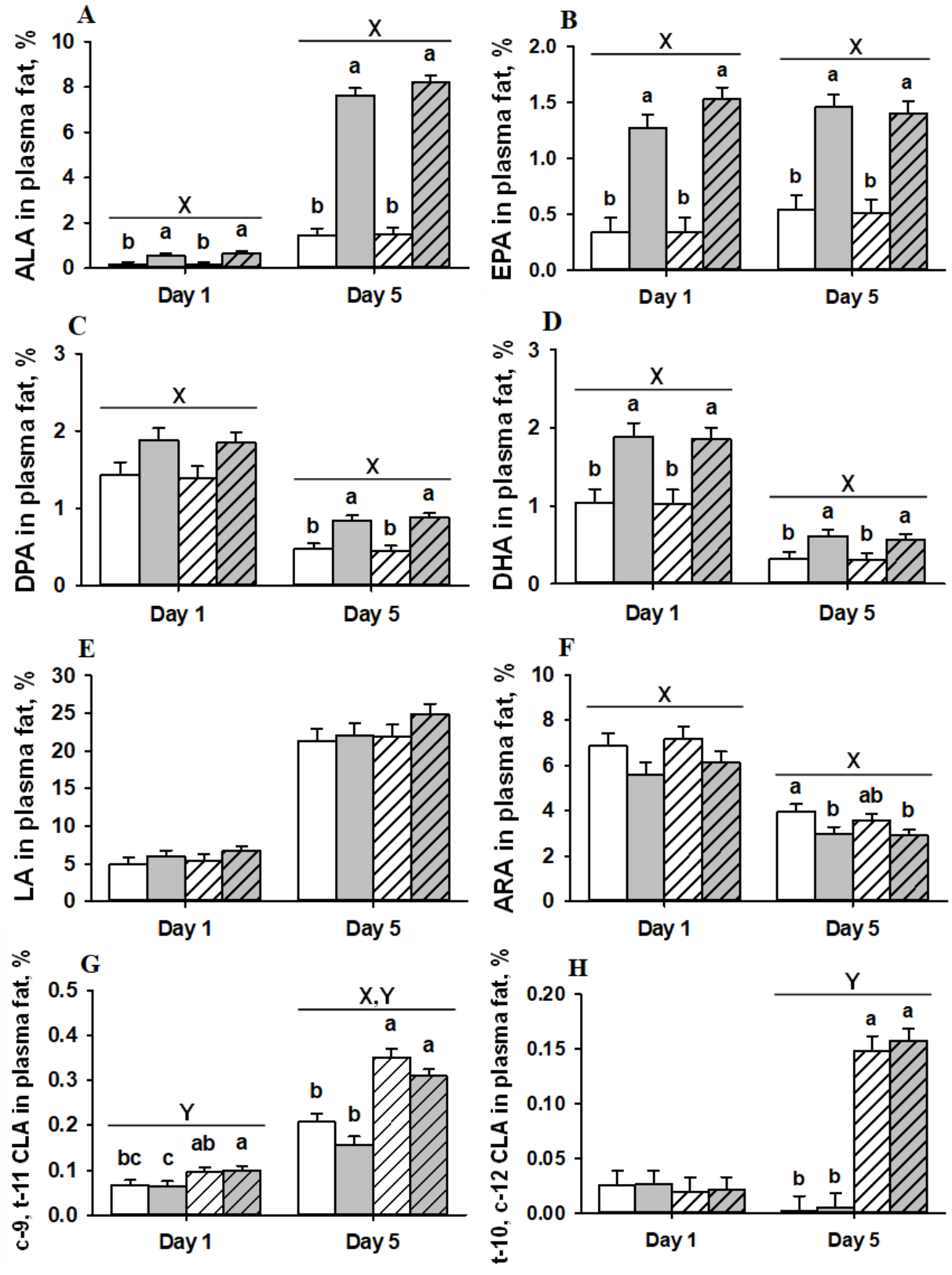


**Figure 2.2: Effects of maternal supplementation with coconut oil (white bars; CTRL; n = 9), linseed and safflower oil (gray bars; EFA; n = 9), Lutalin (BASF SE, Ludwigshafen, Germany; striped white bars; CLA; *cis*-9, *trans*-11 and *trans*-10, *cis*-12; n = 9), or a**

**combination of EFA and CLA (striped gray bars; EFA+CLA; n = 11) on the proportion of α-linolenic acid (ALA; A), eicosapentaenoic acid (EPA; B), docosapentaenoic acid (DPA; C), docosahexaenoic acid (DHA; D), linoleic acid (LA; E), arachidonic acid (ARA; F), *cis*-9, *trans*-11 CLA (*cis*-9, *trans*-11 CLA; G), and *trans*-10, *cis*-12 CLA (*trans*-10, *cis*-12 CLA; H) in plasma of calves on d 1 and 5 of life. Data are presented as LSM ± SE; different letters (a-c) indicate significant differences between groups; X indicates significant differences between EFA- and non-EFA-treated animals; Y indicates significant differences between CLA- and non-CLA-treated animals. A significant effect ($P < 0.05$) was observed for ALA (EFA, time, and EFA × time interaction), EPA (EFA and time), DPA (EFA and time), DHA (EFA, time, and EFA × time interaction), LA (time), ARA (EFA and time), *cis*-9, *trans*-11 CLA (EFA, CLA, time, EFA × time interaction, and CLA × time interaction), and *trans*-10, *cis*-12 CLA (CLA, time, and CLA × time interaction).**

### *2.3.3 Fatty acid composition of plasma lipid fractions*

Maternal treatments did not affect the proportion of TG, PL, CE, or FFA in total plasma lipids (Table 2.3). The largest lipid fraction in plasma was CE, and the other 3 fractions were present at comparable levels. The TG fraction increased ($P < 0.05$) and the FFA fraction decreased ($P < 0.001$) in plasma from d 1 to 5 of life in all groups, whereas the CE and PL fractions indicated an inconsistent pattern over time during the first 5 d of life.

The concentrations of all measured FA in TG, PL, CE, and FFA are presented in Supplemental Tables S2.4 to S2.7. The concentration of ALA increased ($P < 0.001$) from d 1 to 5 and was higher ($P < 0.001$) on d 5 in all lipid fractions except for FFA in calves from EFA-supplemented cows compared with calves from cows receiving no EFA treatment (Figures 2.3A, 2.4A, 2.5A, and 2.6A). The concentration of EPA was higher ($P < 0.05$) on d 1 of life in the CE of calves from EFA- than non-EFA-treated dams. The concentration of EPA increased ($P < 0.05$) in the PL of calves from EFA-treated dams, in the CE of all calves, and in the TG of

EFA+CLA calves and was higher ($P < 0.01$) in all lipid fractions except FFA in EFA- than in non-EFA-treated calves (Figures 2.3B, 2.4B, 2.5B, and 2.6B). Concentrations of DPA and DHA were higher ($P < 0.05$) on d 1 of life in the PL of calves from EFA- than non-EFA-treated dams (Figure 2.4C and 2.4D). Concentrations of DPA and DHA decreased ($P < 0.05$) in TG, PL, and CE but increased ($P < 0.05$) in FFA from d 1 to 5 of life and in PL were higher ($P < 0.05$) on d 5 in calves from EFA- than in calves from non-EFA-treated dams (Figures 2.3C, 2.3D, 2.4C, 2.4D, 2.5C, 2.5D, 2.6C, and 2.6D).

The concentration of LA increased ($P < 0.001$) in all lipid fractions in plasma from d 1 to 5 of life but was only higher ($P < 0.05$) on d 5 in the PL of EFA- than non-EFA-treated calves (Figures 2.3E, 2.4E, 2.5E, and 2.6E). The concentration of ARA decreased ($P < 0.01$) in all lipid fractions except FFA from d 1 to 5 of life and was lower ($P < 0.05$) in PL on d 1 and 5 and in CE on d 5 in calves from EFA- than in calves from non-EFA-treated dams (Figure 2.3F, 2.4F, 2.5F, and 2.6F). Concerning CLA isomers, only *cis*-9, *trans*-11 CLA could be detected in lipid fractions (Figure 2.3G, 2.4G, 2.5G, and 2.6G). The concentration of *cis*-9, *trans*-11 CLA increased ($P < 0.01$) in TG, PL, and FFA from d 1 to 5 and was higher ($P < 0.05$) on d 5 in TG of CLA than in CTRL and EFA, in CE in CLA than in CTRL and EFA+CLA, and in PL in calves for CLA- than in calves from non-CLA treated dams. In FFA, *cis*-9, *trans*-11 CLA was higher ($P < 0.05$) in CTRL than in EFA on d 1 of life.

Plasma FA compositions were mostly similar between female and male calves (data not shown). Only the concentration of eicosatrienoic acid (20:3 *cis*-11, *cis*- 14, *cis*-17, n-3) was lower ($P < 0.01$) in female than male calves ($0.00 \pm 0.00$ vs. $0.03 \pm 0.01$% for female and male calves, respectively).

**Table 2.3: Effects of maternal supplementation with coconut oil (CTRL; n = 9), linseed and safflower oil (EFA; n = 9), Lutalin (BASF SE, Ludwigshafen, Germany; CLA; *cis*-9, *trans*-11 and *trans*-10, *cis*-12; n = 9), or a combination of EFA and CLA (EFA+CLA; n = 11) on the proportion of lipid fractions in the plasma of calves on d 1 and 5 of life[1]**

| | | Maternal supplementation | | | | *P*-value | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Fraction, % | Time[2] | CTRL | EFA | CLA | EFA+CLA | EFA | CLA | EFA × CLA | Time | EFA × time | CLA × time |
| Triglycerides | 1 | 7.59 ± 2.62 | 7.65 ± 2.41 | 7.28 ± 2.53 | 5.92 ± 2.09 | 0.76 | 0.80 | 0.25 | <0.001 | 0.92 | 0.27 |
| | 5 | 14.83 ± 2.99 | 17.43 ± 2.92* | 19.64 ± 2.92* | 16.25 ± 2.47* | | | | | | |
| Phospholipids | 1 | 16.7 ± 3.3 | 16.1 ± 3.0 | 13.4 ± 3.2 | 15.4 ± 2.6 | 0.49 | 0.38 | 0.19 | <0.05 | 0.02 | 0.72 |
| | 5 | 18.0 ± 3.0 | 10.6 ± 2.8 | 12.7 ± 2.9 | 13.1 ± 2.4 | | | | | | |
| Cholesterol esters | 1 | 47.8 ± 2.7 | 44.8 ± 2.5 | 51.8 ± 2.7 | 48.6 ± 2.2 | 0.72 | 0.27 | 0.58 | 0.05 | 0.06 | 0.10 |
| | 5 | 44.2 ± 2.9 | 47.7 ± 2.8 | 45.4 ± 2.8 | 45.6 ± 2.3 | | | | | | |
| Free fatty acids | 1 | 21.8 ± 2.5 | 25.3 ± 2.4 | 21.3 ± 2.4 | 23.7 ± 2.1 | 0.22 | 1.00 | 0.85 | <0.001 | 0.21 | 0.27 |
| | 5 | 11.7 ± 1.7* | 11.2 ± 1.5* | 11.6 ± 1.6* | 13.3 ± 1.3* | | | | | | |

[1]Values are presented as LSM ± SE.

[2]Day of life.

* LSM within a column differs from the LSM on d 1 of life ($P < 0.05$).

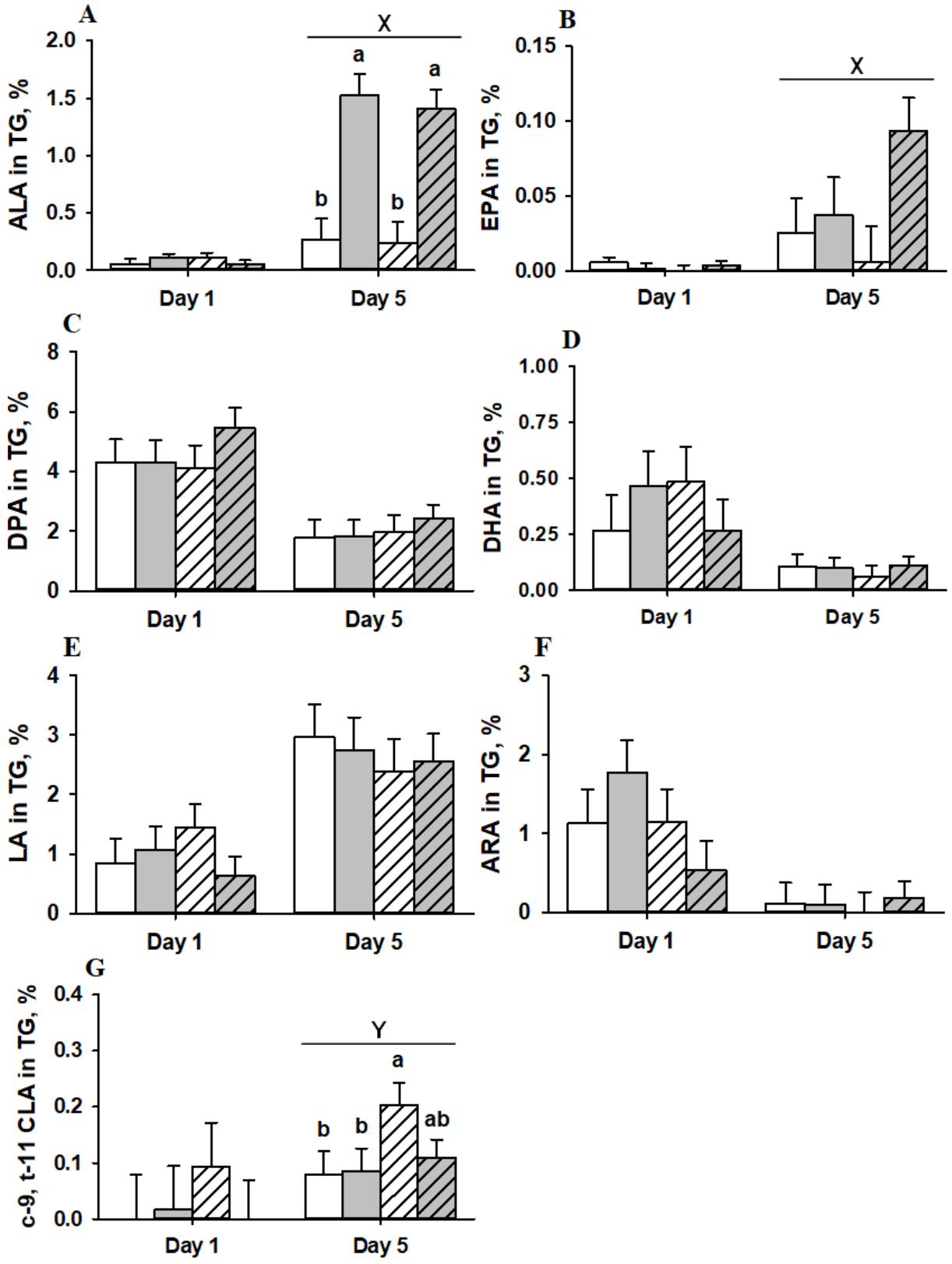


**Figure 2.3: Effects of maternal supplementation with coconut oil (white bars; CTRL; n = 9), linseed and safflower oil (gray bars; EFA; n = 9), Lutalin (BASF SE, Ludwigshafen, Germany; striped white bars; CLA; *cis*-9, *trans*-11 and *trans*-10, *cis*-12; n = 9), or a**

**combination of EFA and CLA (striped gray bars; EFA+CLA; n = 11) on the proportion of α-linolenic acid (ALA; A), eicosapentaenoic acid (EPA; B), docosapentaenoic acid (DPA; C), docosahexaenoic acid (DHA; D), linoleic acid (LA; E), arachidonic acid (ARA; F), and *cis*-9, *trans*-11 CLA (*cis*-9, *trans*-11 CLA; G) in plasma triglycerides (TG) of calves on d 1 and 5 of life. Data are presented as LSM ± SE; different letters (a, b) indicate significant differences between groups; X indicates significant differences between EFA- and non-EFA-treated animals; Y indicates significant differences between CLA- and non-CLA-treated animals. The negative LSM values generated for *cis*-9, *trans*-11 CLA in groups CTRL and EFA+CLA on d 1 and for ARA in group CLA on d 5 were replaced by zero. A significant effect ($P < 0.05$) was observed for ALA (EFA, time, and EFA × time interaction), EPA (time and EFA × time interaction), DPA (time), DHA (time), LA (time), ARA (time), and *cis*-9, *trans*-11 CLA (time).**

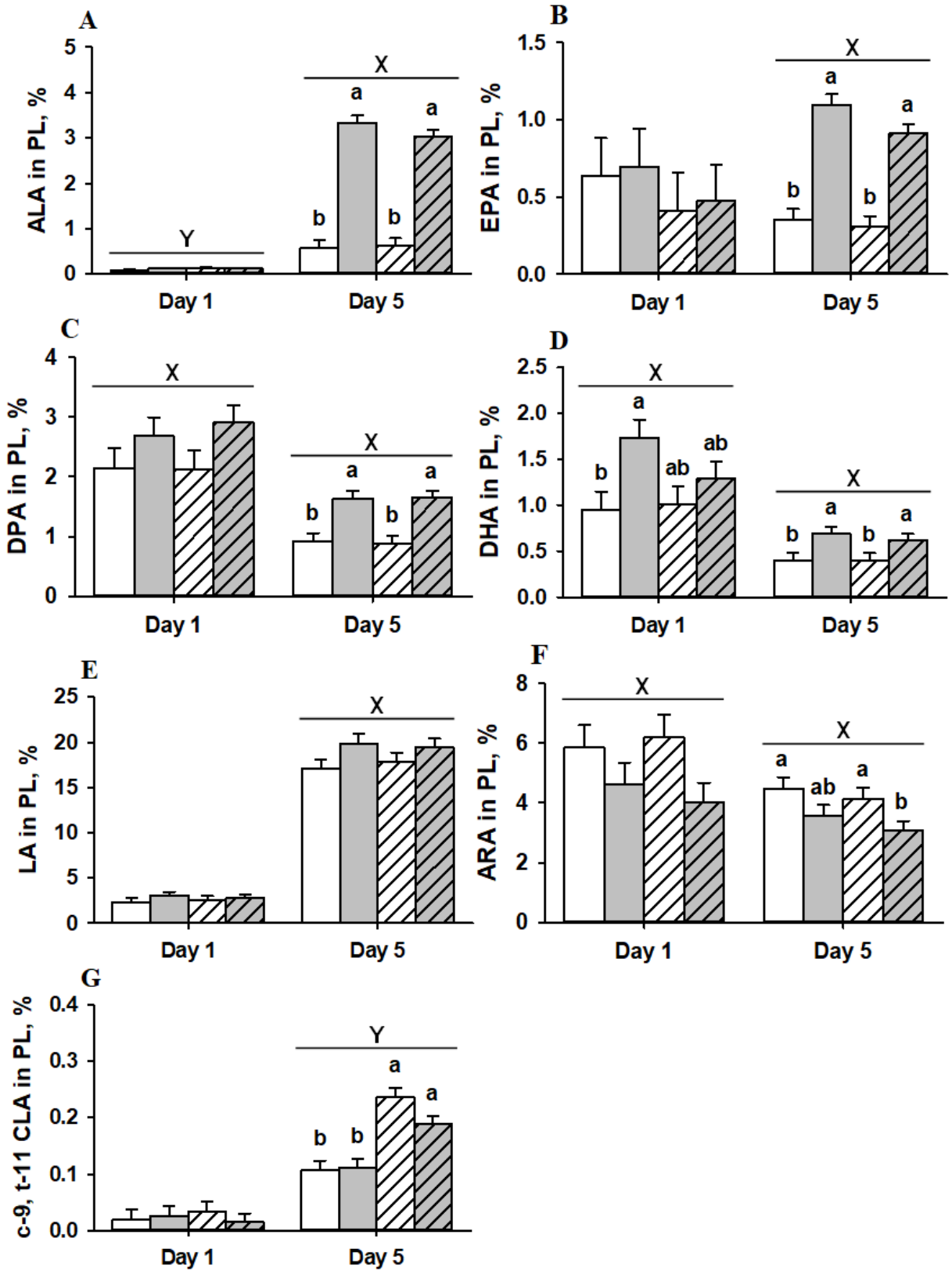


**Figure 2.4: Effects of maternal supplementation with coconut oil (white bars; CTRL; n = 9), linseed and safflower oil (gray bars; EFA; n = 9), Lutalin (BASF SE, Ludwigshafen, Germany; striped white bars; CLA; *cis*-9, *trans*-11 and *trans*-10, *cis*-12; n = 9), or a**

**combination of EFA and CLA (striped gray bars; EFA+CLA; n = 11) on the proportion of α-linolenic acid (ALA; A), eicosapentaenoic acid (EPA; B), docosapentaenoic acid (DPA; C), docosahexaenoic acid (DHA; D), linoleic acid (LA; E), arachidonic acid (ARA; F), and *cis*-9, *trans*-11 CLA (*cis*-9, *trans*-11 CLA; G) in plasma phospholipids (PL) of calves on d 1 and 5 of life. Data are presented as LSM ± SE; different letters (a, b) indicate significant differences between groups; X indicates significant differences between EFA- and non-EFA-treated animals; Y indicates significant differences between CLA- and non-CLA-treated animals. A significant effect ($P < 0.05$) was observed for ALA (EFA, time, and EFA × time interaction), EPA (EFA, and EFA × time interaction), DPA (EFA and time), DHA (EFA and time), LA (EFA and time), ARA (EFA and time), and *cis*- 9, *trans*-11 CLA (CLA, EFA × CLA interaction, time, and CLA × time interaction).**

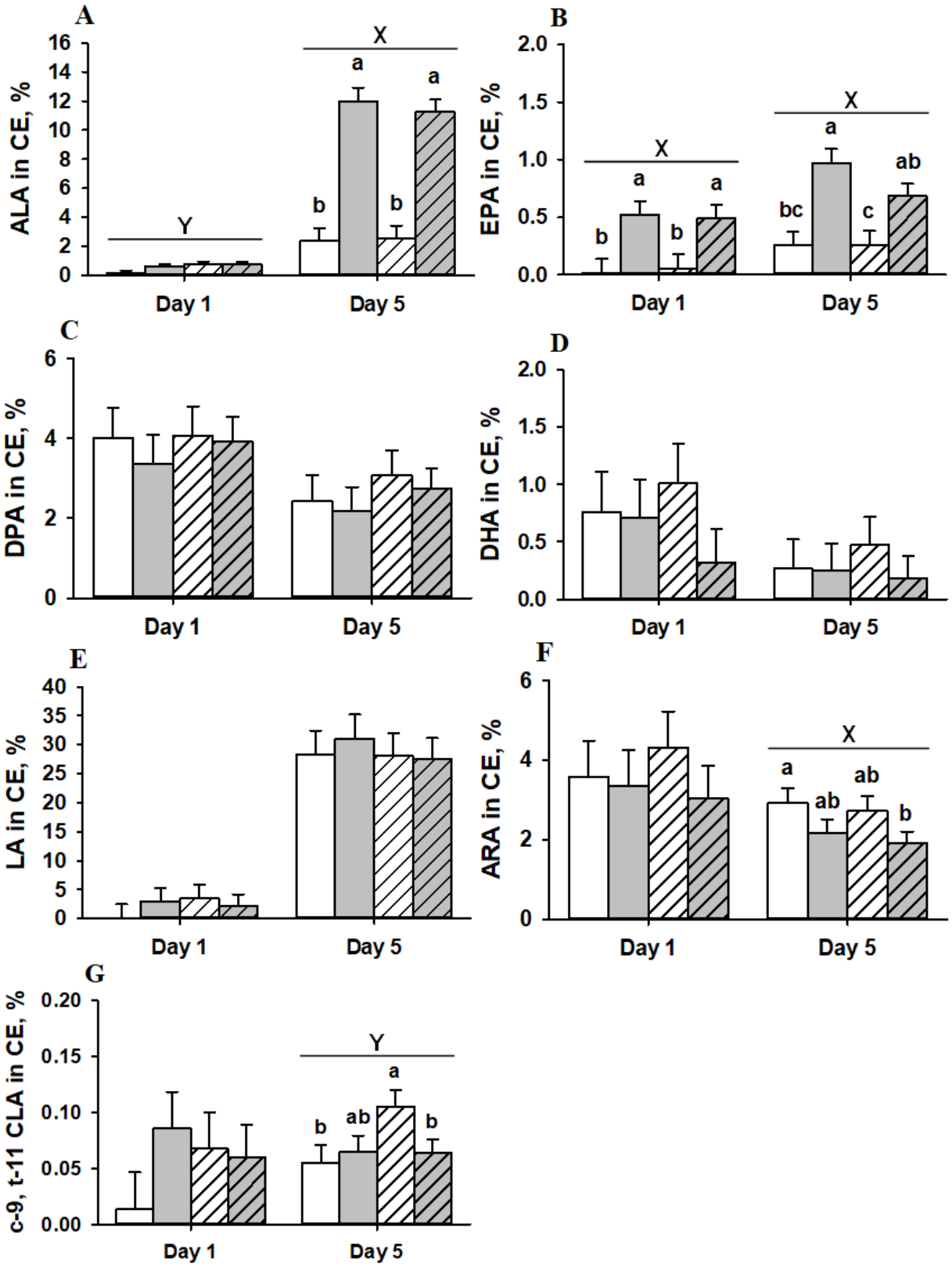


**Figure 2.5: Effects of maternal supplementation with coconut oil (white bars; CTRL; n = 9), linseed and safflower oil (gray bars; EFA; n = 9), Lutalin (BASF SE, Ludwigshafen, Germany; striped white bars; CLA; *cis*-9, *trans*-11 and *trans*-10, *cis*-12; n = 9), or a**

**combination of EFA and CLA (striped gray bars; EFA+CLA; n = 11) on the proportion of α-linolenic acid (ALA; A), eicosapentaenoic acid (EPA; B), docosapentaenoic acid (DPA; C), docosahexaenoic acid (DHA; D), linoleic acid (LA; E), arachidonic acid (ARA; F), and *cis*-9, *trans*-11 CLA (*cis*-9, *trans*-11 CLA; G) in plasma cholesterol esters (CE) of calves on d 1 and 5 of life. Data are presented as LSM ± SE; different letters (a, b) indicate significant differences between groups; X indicates significant differences between EFA- and non-EFA-treated animals; Y indicates significant differences between CLA- and non-CLA-treated animals. The negative LSM value generated for LA in group CTRL on d 1 was replaced by zero. A significant effect ($P < 0.05$) was observed for ALA (EFA, time, and EFA × time interaction), EPA (EFA and time), DPA (time), DHA (time), LA (time), and ARA (time).**

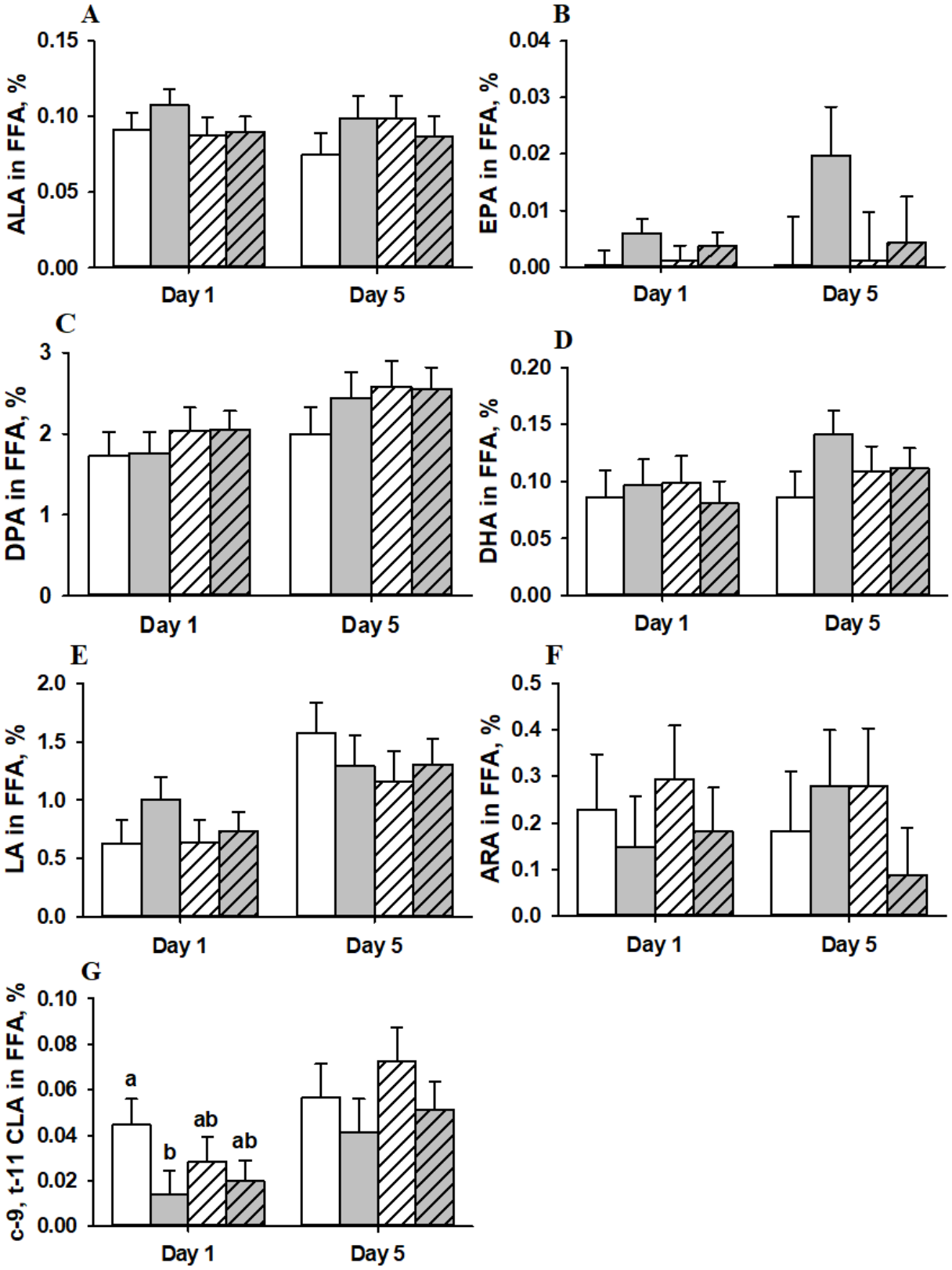


**Figure 2.6: Effects of maternal supplementation with coconut oil (white bars; CTRL; n = 9), linseed and safflower oil (gray bars; EFA; n = 9), Lutalin (BASF SE, Ludwigshafen, Germany; striped white bars; CLA; *cis*-9, *trans*-11 and *trans*-10, *cis*-12; n = 9), or a**

**combination of EFA and CLA (striped gray bars; EFA+CLA; n = 11) on the proportion of α-linolenic acid (ALA; A), eicosapentaenoic acid (EPA; B), docosapentaenoic acid (DPA; C), docosahexaenoic acid (DHA; D), linoleic acid (LA; E), arachidonic acid (ARA; F), and *cis*-9, *trans*-11 CLA (*cis*-9, *trans*-11 CLA; G) in plasma free fatty acids (FFA) of calves on d 1 and 5 of life. Data are presented as LSM ± SE; different letters (a, b) indicate significant differences between groups. A significant effect ($P < 0.05$) was observed for DPA (time), DHA (time), LA (time), and *cis*-9, *trans*-11 CLA (EFA and time).**

## 2.4 Discussion

The study has investigated the perinatal FA status in calves by changing maternal EFA and CLA supply starting in late pregnancy and continuing to the colostrum and transition milk period. Since maternal EFA and CLA treatments were performed by abomasal infusion of the dams, no rumen degradation of the supplied FA occurred (Vogel et al., 2020). The study revealed the importance of colostral and transition milk transfer of ALA and LA to their offspring but in addition pointed at a significant placental transfer of n-3 and n-6 FA metabolites to the fetus.

### *2.4.1 Effects on gestation length, colostrum and transition milk chemical composition, and calf performance*

The results of the present study indicate an effect of maternal FA supply on colostrum composition. The lower DM content in colostrum from the first milking after calving of CLA-treated cows than that of non-CLA-treated cows was probably a consequence of a lower protein concentration in the colostrum of CLA-treated cows. In a recent study, a comparable CLA treatment resulted in a lower milk protein concentration in mid- to late-lactating dairy cows, and a repartitioning of body protein by the CLA treatment was discussed (Haubold et al., 2020). However, it is not yet known whether CLA treatment affects milk protein synthesis at the beginning of lactation. Many proteins in colostrum, such as immunoglobulins and growth factors, are stored, but not synthesized, by the mammary gland during the dry period (Grosvenor et al., 1993; Baumrucker and Bruckmaier, 2014). Therefore, a CLA effect on systemic whole-body protein synthesis cannot be excluded. However, the lower protein concentration in colostrum from the first milking in the present study did not result from a reduced IgG concentration because the IgG1 and IgG2 concentrations in the first colostrum did not differ among treatment groups [K. L. Uken, T. Stefaniak (Wroclaw University of Environmental and Life Science, Poland), and H. M. Hammon, unpublished observation].

Interestingly, EFA treatment increased the lactose concentration but decreased the fat concentration in milk from several milkings after calving, leading to lower fat concentrations in the colostrum of EFA supplemented cows. A reduced milk fat content was repeatedly observed in previous studies when cows received linseed products. However, in those studies, milk fat depression was presumably caused by the formation of *trans* FA from ruminal biohydrogenation of EFA (Shingfield et al., 2010; Moallem, 2018). We applied EFA directly into the abomasum to prevent ruminal biohydrogenation. Thus, a reduced de novo FA synthesis in the mammary gland, indicated by a lower proportion of middle-chain FA in the first colostrum (Vogel et al., 2020), was the result of a greater availability of preformed FA by EFA infusion. The reduced de novo FA synthesis in the mammary gland due to EFA infusion was obviously responsible for the observed milk fat decrease in the mammary tissue of EFA-treated cows, as previously indicated in cows supplemented with linseeds or canola oil (Chelikani et al., 2004; Lerch et al., 2015). The decrease in milk fat under the EFA treatment was not associated with treatment effects on DMI or the plasma nonesterified FA concentration in cows around calving (Vogel et al., 2020), indicating that the increased availability of long-chain FA by the EFA infusion per se was sufficient to inhibit de novo FA synthesis and reduce the milk fat concentration.

Supplementation with CLA did not reduce milk fat during the first milkings after calving. A significant milk fat reduction by CLA treatment in these cows was observed during late lactation and again after calving at the end of the first week, although the CLA treatment comprised infusion from wk 9 before calving without interruption but with half the dose during the dry period (Vogel et al., 2020). The diminishing effect of CLA treatment on milk fat depression immediately after calving is well known from the literature (Castaneda- Gutierrez et al., 2005; Odens et al., 2007; Hötger et al., 2013). Since CLA primarily inhibits de novo FA synthesis, which is already reduced immediately after birth due to low feed intake and limited

acetate availability, the milk fat decrease with the CLA treatment during the first days after calving was less effective.

The availability of fat and energy on d 1 and 3 of life was lower for calves whose dams received the EFA treatment. In addition, maternal EFA supplementation seemed to prolong gestation, as indicated by a trend toward longer gestation length for EFA-supplemented cows. Prolonging effects of n-3 FA on gestation were repeatedly described in the literature and are associated with increased EPA availability that leads to a lower synthesis of series-2 prostaglandins (Olsen et al., 1992; Abayasekara and Wathes, 1999; Pickard et al., 2008). Although maternal FA supplementation partly modulated gestation time and nutrient availability from colostrum and transition milk, maternal FA supplementation did not affect birth weight or body growth until d 5 of life. The experimental period in the current study was too short to estimate growth effects in calves. Nevertheless, there might be effects on the postnatal growth regulation by the somatotropic axis in these calves. At least, the CLA and EFA+CLA treatments affected parameters of the somatotropic axis in dairy cows during the transition period (Vogel et al., 2021). Feeding goats or ewes variable amounts of n-3 FA during late gestation had no effect on the birth weight of the offspring in previous studies (Duvaux-Ponter et al., 2008; Coleman et al., 2018; Nickles et al., 2019). Nevertheless, there are indications that ADG can be improved when calves are fed increased quantities of ALA alone or both ALA and LA (Hill et al., 2009; Garcia et al., 2014b). However, calves in those studies were only investigated during the first 30 d of life. Thus, a longer observation period might be required to evaluate whether an elevated maternal EFA supply by milk can improve growth performance.

### *2.4.2 Effects on FA profiles in blood plasma immediately after birth*

Providing supplementation of EFA containing high amounts of ALA to dams during late gestation increased ALA concentration in plasma fat of cows 4.5- fold (2.29 ± 0.41% and 10.20

± 0.41% for CTRL and EFA cows, respectively, at d 42 before calving; Gnott et al., 2020). The increase of plasma ALA corresponded to changes of ALA in milk fat when cows were on pasture with fresh grass instead of receiving a corn silage-based TMR (Kelly et al., 1998; Kay et al., 2005; Couvreur et al., 2006). As a consequence of maternal ALA supplementation during late gestation in the present study, there was a slight increase of plasma ALA concentration in calves before first colostrum intake. However, EFA supplementation to the dams barely caused significant changes in ALA concentrations in individual plasma lipid fractions of the calves directly after birth. This illustrates the low transfer of ALA through the placenta to the bovine fetus. This finding supports previous investigations on the transfer of ALA from the mother to the fetus during late gestation, although the treatment times and concentrations of ALA were slightly different (Moallem and Zachut, 2012).

Even though the treatment effect of maternal EFA supply on fetal ALA concentration was low, the maternal EFA supply resulted in elevated proportions of EPA and DHA in plasma fat of EFA and EFA+CLA calves at birth. The n-3 metabolites were especially increased in the CE fraction (EPA) and PL fraction (DHA) of the blood plasma. In ruminants, most of the PUFA in plasma are localized in the CE and PL fractions (Christie, 1981; Innis, 2005; Palmquist, 2010). Fetal n-3 synthesis by elongation and desaturation of ALA cannot be excluded (Shand et al., 1978; Elmes et al., 2004), but the present study suggests that the n-3 FA metabolites in fetal blood most likely resulted from the dam through placental transfer. All measured n-3 FA increased in maternal blood plasma after EFA or EFA+CLA treatment, and plasma concentrations of EPA, DPA, and DHA were elevated in EFA-supplemented dams during late gestation and around calving, as shown in Supplemental Table S2.3 and illustrated in more detail by Gnott et al. (2020). α-Linolenic acid can serve as a precursor for the synthesis of n-3 FA metabolites in the placenta, which possesses significant desaturase activity in ruminants (Duvaux-Ponter et al., 2008; Garcia et al., 2014a). In addition, the enrichment of DHA in plasma fat of the calves immediately after birth was approximately 8-fold higher than in plasma

fat of their dams, especially when comparing calves and cows of the EFA treatment (Gnott et al., 2020). Even though placental transfer of PUFA is low, an increased proportion of DHA but not ALA in the plasma fat of newborn calves was already observed by Moallem and Zachut (2012). A selective transfer of DHA by the placenta was also reported in humans (Haggarty, 2002; Herrera, 2002; Hanebutt et al., 2008). Our results suggest that a similar mechanism must be active in the bovine placenta for DHA to be enriched in the fetal plasma fat, although histological compositions of human and bovine placentas differ greatly. On the other hand, plasma desaturase indices were low in calf plasma fat immediately after birth, which may indicate that DHA synthesis was not elevated by the fetus.

Numerous studies have indicated that LA can pass the ruminant placenta as well (Noble et al., 1978a; Garcia et al., 2014a; Salehi and Ambrose, 2017). The proportion of LA in plasma fat in the calves before first colostrum feeding was lower than that in the dams, but the ARA concentration was higher in the plasma fat of the calves than of the dams at calving. This finding is consistent with results on DHA supply, as discussed above, and placental n-6 FA transport in fetal sheep (Noble et al., 1982). Interestingly, there was only a numerically lower ARA concentration in the neonatal plasma fat after EFA treatment, even though the maternal ARA proportion in plasma fat and the neonatal Δ5 desaturase index were lower due to EFA treatment. Whether the reduction of the LA/ALA ratio by EFA supplementation affects Δ5 desaturase activity and impairs the synthesis and transfer of n-6 FA derivatives in the bovine fetus is not yet known; however, a reduction of the LA/ALA ratio in the diet seems to lead to a preferential desaturation of n-3 instead of n-6 FA in the livers of rats (Geiger et al., 1993).

The supply of *cis*-9, *trans*-11 CLA of the fetal calf may be enhanced if dams receive this CLA isomer, as suggested by the higher percentages of *cis*-9, *trans*-11 CLA in plasma fat of calves from CLA-treated dams directly after birth. However, the low correlation between *cis*-9, *trans*-11 CLA percentages in dam and calf plasma might indicate a more limited placental transfer of *cis*-9, *trans*-11 CLA than of ALA. Accordingly, Dänicke et al. (2012) found only a

trend toward higher percentages of *cis*-9, *trans*-11 CLA in erythrocyte lipids of calves, whose dams received a supplement containing *cis*-9, *trans*-11 CLA during early gestation. Moreover, the authors did not detect *trans*-10, *cis*-12 CLA in the erythrocyte lipids of calves, which was also supplemented during early gestation (Dänicke et al., 2012). In the present study, *trans*-10, *cis*-12 CLA was not enhanced in calves at birth after maternal *trans*- 10, *cis*-12 CLA treatment during late gestation. It is unclear why maternal supplementation with the *trans*- 10, *cis*-12 isomer failed to increase its proportion in the plasma fat of neonatal calves. The lack of a relationship between the percentage of this isomer in maternal and neonatal plasma fat at birth might indicate that an altered maternal *trans*-10, *cis*-12 CLA status was not transferred to the fetus via the placenta.

### *2.4.3 Effects on FA profiles in blood plasma after milk feeding*

Supplementation of ALA by the EFA treatment resulted in a 19-fold increase of ALA in colostrum fat of EFA-cows when compared with CTRL cows (0.07 ± 0.11% and 1.34 ± 0.10% for CTRL and EFA, respectively; Supplemental Table S2.3; Vogel et al., 2020). Concentration of ALA in colostrum fat was slightly higher than in milk fat of cow fed fresh grass or on pasture (ALA in milk fat: 0.7-1.12%; Kelly et al., 1998; Kay et al., 2005; Couvreur et al., 2006), but in a recent study a greater EFA content in colostrum than mature milk in dairy cows was shown (O’ Callaghan et al., 2020). Therefore, the FA supply via colostrum and transition milk determined the ALA status of the neonatal calf in the present study. Especially in calves of the EFA and EFA+CLA groups, high percentages of ALA in plasma fat, particularly in the TG, PL, and CE fractions, were accomplished after feeding of colostrum and milk during the first 5 d of life. In CE, ALA proportions up to 12% were observed after feeding on colostrum and milk from EFA-supplemented dams. Moreover, the correlation coefficients of ALA and LA were higher for the relationship of FA percentages between colostrum and calf plasma than between dam and calf plasma in the present study. This observation supported the assumption that the

placental transfer of EFA is under tighter control than transfer via the mammary gland (Garcia et al., 2014a). Concordantly, Garcia et al. (2016) observed that the addition of LA to milk replacer modified plasma FA in calves to a greater degree than maternal supplementation with the FA during late gestation. An elevated LA supply via the mammary gland was also observed in sheep (Noble et al., 1978b). Furthermore, the percentages of LA and ALA are already increased in the first milking after parturition if the dam is supplemented with these FA (Santschi et al., 2009; Salehi et al., 2016). Consequently, feeding on colostrum and transition milk from EFA-supplemented dams improved the availability of EFA for young calves.

In contrast to ALA and LA, the percentage of n-3 and n-6 metabolites, such as DPA, DHA, and ARA, decreased from d 1 to 5 of life in plasma fat and especially in plasma TG, PL, and CE. Furthermore, the proportions of PL and CE, where most of the n-3 and n-6 FA are stored (Christie, 1981; Palmquist, 2010), did not change from d 1 to 5. Consequently, the enrichment of DPA, DHA, and ARA in lipid fractions could not be maintained by colostrum feeding, as was the case for ALA and LA. Obviously, there is a preferred transport of ALA and LA via colostrum and milk feeding in neonatal calves, but this was not observed for n-3 and n-6 metabolites. Only the EPA concentration did not decrease during the first 5 d of life in calves. This finding was surprising because the DPA concentration in colostrum indicated a higher enrichment of DPA than EPA in milk fat of EFA and EFA+CLA cows (Vogel et al., 2020). On the other hand, DHA was not detected in the milk fat of the cows (Vogel et al., 2020). Nevertheless, percentages of EPA, DPA, and DHA were higher in the plasma fat and especially in the PL fraction of calves whose dams received the EFA and EFA+CLA treatment than in calves from CTRL- and CLA-supplemented dams. However, it might be reasonable to supply neonatal calves with fish oil to increase availability of n-3 FA metabolites such as EPA and DHA, as recently shown by Opgenorth et al. (2020).

As mentioned above, n-3 and n-6 FA are primarily stored in the PL and CE lipid fractions (Christie, 1981; Palmquist, 2010). In the CE fraction, EPA clearly increased on d 5 only in EFA

calves. Overall, the importance of placental and mammary transfer may depend on individual FA supplies, with milk transfer being critical for the neonatal ALA and LA supplies. The neonatal status of n-3 and n-6 FA metabolites, especially DHA and ARA, obviously depends more on placental than mammary FA transfer. The present study did not allow us to distinguish between exogenous supplies and endogenous synthesis of n-3 and n-6 FA metabolites in the 5-d-old calves, but we assume that the endogenous syntheses were low due to the low Δ6 desaturase activity in neonatal ruminants (Shand et al., 1978; Doreau et al., 2011).

In contrast to the limitations of placental CLA transport, the *cis*-9, *trans*-11 and *trans*-10, *cis*-12 CLA from CLA-supplemented dams were transferred to calves via colostrum and milk. The fact that *cis*-9, *trans*-11 CLA but not *trans*-10, *cis*-12 CLA increased in plasma fat of calves born by dams not treated with CLA mirrored the higher concentrations of *cis*-9, *trans*-11 than *trans*- 10, *cis*-12 CLA in colostrum and milk (Vogel et al., 2020). In calves from CLA-treated cows, both CLA isomers increased to a greater extent compared with those in calves without maternal CLA treatment. The additional increase due to CLA supplementation seemed to be comparable between the 2 CLA isomers. Due to the lower total *trans*-10, *cis*-12 CLA concentration in calves, this isomer was not detectable in individual lipid fractions of neonatal blood plasma, whereas *cis*-9, *trans*-11 CLA was elevated in calf TG, PL, and CE fractions after feeding on colostrum and milk from dams of the CLA groups. This omnidirectional incorporation of additionally available CLA into several lipid fractions was also observed in a previous study, in which supplemented *cis*-9, *trans*-11 CLA was channeled into several lipid classes of rat liver lipids (Banni et al., 2001).

## Conclusions

The results of the present study suggest that an increased maternal EFA and CLA supply during late lactation improved the EFA and CLA status in the neonatal calves, primarily by an elevated supply via colostrum and transition milk feeding. The majority of EFA were stored in

the PL and CE plasma fractions. In contrast to ALA and LA, n-3 and n-6 metabolites such as DHA and ARA were enriched in calves immediately after birth, supporting the concept of a preferred placental transfer of DHA and ARA in bovines. The FA supplementation of dams during late lactation indicated minor effects on gestation length and nutrient composition in colostrum and transition milk, slightly affecting nutrient intake after birth, but did not influence fetal and neonatal growth performance.

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# CHAPTER 3

# Impact of maternal supplementation with essential fatty acids and conjugated linoleic acid on metabolic and endocrine development of neonatal calves

**K. L. Uken[1], L. Vogel[1], M. Gnott[1], S. Görs[1], C. T. Schäff[1], A. Tuchscherer[1], A. Hoeflich[1], J. M. Weitzel[1], E. Kanitz[1], A. Tröscher[2], H. Sauerwein[3], R. Zitnan[4], R. M. Bruckmaier[5¶], J. J. Gross[5¶], W. Liermann[1], and H. M. Hammon[1*]**

[1]Leibniz Institute for Farm Animal Biology (FBN), 18196 Dummerstorf, Germany

[2]BASF SE, 68623 Lampertheim, Germany

[3]Institute of Animal Science, Physiology Unit, University of Bonn, 53115 Bonn, Germany

[4]Institute of Nutrition, Research Institute for Animal Production Nitra, National Agricultural and Food Centre, 95141 Luzianky, Slovakia

[5]Veterinary Physiology, Vetsuisse Faculty, University of Bern, 3001 Bern, Switzerland

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[1]Corresponding author: hammon@fbn-dummerstorf.de

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# 3. Impact of maternal supplementation with essential fatty acids and conjugated linoleic acid on metabolic and endocrine development of neonatal calves


## Abstract

We tested the hypothesis that the maternal supply of essential fatty acids (EFA), especially α-linolenic acid, and conjugated linoleic acid (CLA), affects glucose metabolism, the endocrine regulation of energy metabolism and growth, and the intestinal development of neonatal calves. We studied calves from dams that received an abomasal infusion of 76 g/d coconut oil (CTRL; n = 9), 78 g/d linseed oil and 4 g/d safflower oil (EFA; n = 9), 38 g/d Lutalin (BASF SE) containing 27% *cis*-9, *trans*-11 and *trans*-10, *cis*-12 CLA (CLA; n = 9), or a combination of EFA and CLA (EFA+CLA; n = 11) during the last 63 d of gestation and early lactation. Calves received colostrum and transition milk from their own dam for the first 5 d of life. Insulin-like growth factor (IGF)-I, leptin, and adiponectin concentrations were measured in milk. Blood samples were taken before first colostrum intake, 24 h after birth, and from d 3 to 5 of life before morning feeding to measure metabolic and endocrine traits in plasma. On d 3 of life, energy expenditure was evaluated by a bolus injection of $NaH^{13}CO_3$ and determination of $CO_2$ appearance rate. On d 4, additional blood samples were taken to evaluate glucose first-pass uptake and $^{13}CO_2$ enrichment after [$^{13}C_6$]-glucose feeding and intravenous [6,6-$^2H_2$]-glucose bolus injection, as well as postprandial changes in glucose, nonesterified fatty acids (NEFA), insulin, and glucagon. On d 5, calves were killed 2 h after feeding and samples of small intestinal mucosa were taken for histomorphometric measurements. The concentrations of IGF-I, adiponectin, and leptin in milk decreased during early lactation in all groups, and the concentrations of leptin in first colostrum was higher in EFA than in CTRL cows. Plasma glucose concentration before first colostrum intake was higher in EFA calves than in non-EFA

calves and was lower in CLA calves than in non-CLA calves. Plasma IGF-I concentration was higher on d 1 before colostrum intake in EFA calves than in EFA+CLA calves and indicated an overall CLA effect, with lower plasma IGF-I in CLA than in non-CLA calves. Postprandial NEFA concentration was lowest in EFA and CLA calves. The postprandial rise in plasma insulin was higher in EFA than in non-EFA calves. Plasma adiponectin concentration increased from d 1 to d 2 in all groups and was higher on d 3 in CLA than in non-CLA calves. Plasma leptin concentration was higher on d 4 and 5 in EFA than in non-EFA calves. Maternal fatty acid treatment did not affect energy expenditure and first-pass glucose uptake, but glucose uptake on d 4 was faster in EFA than in non-EFA calves. Crypt depth was lower, and the ratio of villus height to crypt depth was higher in the ilea of CLA than non-CLA calves. Elevated plasma glucose and IGF-I in EFA calves immediately after birth may indicate an improved energetic status in calves when dams are supplemented with EFA. Maternal EFA and CLA supplementation influenced postprandial metabolic changes and affected factors related to the neonatal insulin response.

**Key words:** calf, essential fatty acids, conjugated linoleic acid, neonatal energy metabolism

## 3.1 Introduction

Due to their indispensability for mammalian growth and development, and mammals' inability to synthesize them, linoleic acid (18:2 *cis*-9, *cis*-12) and α-linolenic acid (18:3 *cis*-9, *cis*-12, *cis*-15) are classified as essential fatty acids (EFA; Burr and Burr, 1930; Neuringer et al., 1986). These molecules serve as structural components of membranes, acting as ligands that regulate transcription factors, and they provide precursors to other molecules that modulate cell metabolism (e. g., in the neural cell during fetal maturation; Innis, 2005). Conjugated linoleic acids, which are predominantly formed in the rumen from EFA and also in the mammary tissue of dairy cows (Bauman et al., 2000), can also act as ligands for transcription factors, modulate the synthesis of lipids (Moya-Camarena et al., 1999; Harris et al., 2001), and influence

metabolic processes in dairy cows (Baumgard et al., 2000; Odens et al., 2007; Hötger et al., 2013). The cow’s supply of these fatty acids has changed as a result of replacing pasture and fresh grass with diets based on corn silage in modern dairy cow nutrition. Compared with pasture, corn silage provides high amounts of linoleic acid, but low amounts of α-linolenic acid (Ferlay et al., 2006). Furthermore, less CLA is synthesized in cows fed corn silage-based diets than in those fed a pasture or fresh grass diet (Kay et al., 2005; Couvreur et al., 2006). During gestation and via the intake of colostrum and milk, the maternal supply of EFA can be transferred to the calf (Garcia et al., 2014). The first results of the present study showed increased n-3 fatty acid and CLA concentrations, as well as decreased n -6: n -3 fatty acid status in colostrum and in the blood plasma of calves when dams were supplemented with EFA (mainly n-3 fatty acids provided by linseed oil) and CLA (*cis*- 9, *trans*-11 CLA and *trans*-10, *cis*-12 CLA) during late gestation and early lactation (Vogel et al., 2020; Uken et al., 2021).

The maternal supply of α-linolenic acid and linoleic acid can modulate development and the metabolic processes in calves (Abuelo, 2020). For instance, maternal n-3 supplementation during gestation and lactation might improve intestinal glucose absorption by increasing the number of intestinal glucose transporters, as has been shown in pigs (Gabler et al., 2007). However, in rats an elevated ratio of n-6 to n-3 in the maternal diet during gestation and lactation favored glucose uptake in the jejunum of the offspring (Jarocka-Cyrta et al., 1998). Furthermore, calves fed milk replacer that provided increased amounts of linoleic and α-linolenic acid showed elevated growth performance, had higher glucose concentrations, and tended to have higher IGF-I concentrations in plasma (Garcia et al., 2014). In contrast, Hill et al. (2009) and Esselburn et al. (2013) observed a linear decrease in serum glucose and urea in calves when intake of α-linolenic acid through linseed oil or commercial product was increased in the starter feed or milk replacer. Interestingly, n-3 fatty acid supplementation improved insulin sensitivity in cattle (Pires et al., 2008), and the gene expression of enzymes related to gluconeogenesis may be under the control of long-chain fatty acids (White et al., 2011). The

treatment of bovine kidney cells with EFA affected their energy metabolism and fatty acid oxidation (Boesche and Donkin, 2020), and α-linolenic acid treatment of bovine kidney cells increased the activity of pyruvate carboxylase promoter 1 (Boesche and Donkin, 2021), a key enzyme that regulates gluconeogenesis in cattle (Donkin, 2016). Furthermore, intestinal morphology might be affected by an enhanced maternal EFA supply during gestation and in the early postnatal phase, as demonstrated in the ilea of piglets whose dams received diets including linseed oil or lard (Boudry et al., 2009). Authors showed reduced villus growth and crypt depth in the ilea of piglets at birth when sows were fed linseed oil during gestation, resulting in elevated n-3 fatty acid status. In contrast, neonatal energy metabolism seems to be less affected by maternal CLA supply in cattle. Petzold et al. (2014) did not find a neonatal metabolic response when cows were fed 100 g/d CLA starting at 3 wk before calving.

The present study aimed to investigate the effects of increased maternal supply of EFA and CLA during late gestation and early lactation on neonatal energy metabolism and intestinal mucosal growth in calves. We hypothesized that an enhanced maternal EFA and CLA supply would promote glucose metabolism, especially intestinal glucose uptake, by influencing endocrine factors related to energy metabolism, growth, and intestinal development in neonatal calves during their first 5 d of life.

## 3.2 Materials and methods

The experimental procedures were conducted according to German animal-care guidelines and were approved by the authorities of Mecklenburg-West Pomerania, Germany (Landesamt fur Landwirtschaft, Lebensmittelsicherheit und Fischerei; LALLF M-V/ TSD/7221.3-1-052/15).

### *3.2.1 Animals, experimental design, and husbandry*

The present study examined the offspring of 37 German Holstein cows that were evaluated in a comprehensive experiment that investigated the effects of EFA and CLA during late

pregnancy and early lactation (Vogel et al., 2020, 2021). The planned number of animals and group size were chosen to ensure a type I error probability of 0.05 and a type II error probability of 0.20 (i.e., power of 0.8).

Briefly, dams received corn silage-based diets with a low EFA content, providing particularly low amounts of n-3 fatty acids (1.4 and 9.5 g of n-3 and n-6 fatty acids per kg of DM) from the middle of the second lactation (wk 22 antepartum) to the 3rd lactation (wk 9 postpartum). Dams were assigned to 1 of 4 treatments based on milk yield and BW: the control group (CTRL), supplemented with 76 g/d coconut oil (Bio- Kokosöl #665; Kräuterhaus Sanct Bernhard KG); the EFA group, supplemented with EFA in the form of 78 g/d linseed oil (DERBY Leinöl #4026921003087; Derby Spezialfutter GmbH) and 4 g/d safflower oil (Gefro Distelöl; Gefro Reformversand Frommlet KG), providing a fatty acid ratio of 1:3 (n -6: n -3) in the supplement mixture; the CLA group, supplemented with 38 g/d Lutalin (*cis*-9, *trans*-11 and *trans*-10, *cis*-12 CLA, 10 g/d each; BASF SE); or the EFA+CLA group, which received a combination of the EFA and CLA supplements (78 g/d linseed oil + 4 g/d safflower oil + 38 g/d Lutalin). As previously reported by Vogel et al. (2020), the CTRL supplement provided less than 1.4 g/d EFA. The EFA supplement provided 39.9 g/d α-linolenic acid and 14.9 g/d linoleic acid. To compensate for the vitamin E that naturally occurs in linseed oil, we added vitamin E to the CTRL and CLA supplements. Cows were fitted with rumen cannulas and abomasal infusion lines, and all supplements were applied via abomasal injection to avoid ruminal biohydrogenation. Fatty acids were infused using 60 mL catheter-tip syringes twice a day (2 equal portions) at 0700 and 1630 h. All supplements were liquified by heating to 38°C to allow infusion (Vogel et al., 2020). Supplementations started 63 d before expected parturition and continued into early lactation. During the dry period, comprising the last 6 wk of gestation, the amount of supplement was halved. For technical reasons, the calving periods were subdivided into 5 consecutive blocks, with 7 to 8 calves born per block.

The experimental design of the calf study was recently presented in detail in a companion paper (Uken et al., 2021). In total, 38 calves were investigated: 9 in the CTRL group (5 male, 4 female), 9 in the EFA group (4 male, 5 female), 9 in the CLA group (1 male, 8 female), and 11 in the EFA+CLA group (4 male, 7 female; 9 single births and 1 pair of twins: 1 male, 1 female). All calves were separated from their dam immediately after birth. During the experiment, which comprised their first 5 d of life, calves were housed in a climate-controlled room at 19°C in single boxes on straw bedding and with free access to water. The calves were fed colostrum and transition milk from their own dam. If the first colostrum quantity of a dam was insufficient, the required volume was replenished with colostrum from a cow in the same treatment group to ensure a consistent fatty acid supply within treatment groups; this happened 4 times (CTRL and EFA groups). First colostrum was fed 2.5 ± 1.7 h after birth on average. Calves were fed with nipple bottles, and calves that refused milk intake were tube-fed to ensure similar intake. Calves were fed colostrum from the first milking in amounts of 10% BW during the first 24 h after birth, divided into 2 meals. Colostrum from the second milking after calving was fed only if the amount of the first colostrum was not sufficient for the second meal. On d 2 (24 h after birth and before beginning of d 3 of life), calves were fed transition milk from milking 3 after calving. Feed allowance was 6% of BW on d 2 to ensure that all calves received the same amount of transition milk before d 3 of life, irrespective of whether they were born in the morning or afternoon the day before. From d 3 onwards, the calves were fed transition milk from the 5th, 7th, and 9th milking after calving at 12% BW/d, divided into 2 meals (morning and evening). The exact nutrient intakes of the calves are presented in a companion paper (Uken et al., 2021; Chapter 2, Table 2.1). Individual colostrum and transition milk samples from the daily morning and afternoon milkings were collected and stored at -20°C until analysis for IGF-I, leptin, and adiponectin.

### *3.2.2 Milk analyses*

Milk serum was obtained in defatted colostrum and transition milk by double centrifugation at 4°C (15 min at 1,000 × g). Then, the infranatant was centrifuged again (30 min at 20,000 × g). The resulting infranatant was used for IGF-I determination by RIA (Vicari et al., 2008). Intra- and interassay coefficients of variation (CV) for IGF-I RIA were less than 10 and 15%, respectively. Adiponectin in milk was measured by ELISA (Mielenz et al., 2013; Kesser et al., 2015). The intra- and interassay CV were 8.0 and 9.3%, respectively. Milk leptin concentration was determined by ELISA (Sauerwein et al., 2004), and the intra- and interassay CV were 8.1 and 11.4%, respectively. Concentrations of IGF-I, adiponectin, and leptin in colostrum and transition milk in grams per kilogram were computed from concentrations in grams per liter by correcting for the density of milk from the respective milking, according to data from Madsen et al. (2004).

### *3.2.3 Blood sampling and analyses*

Blood was sampled from the jugular vein by venipuncture using evacuated tubes containing $K_3$EDTA (1.2-2 mg $K_3$EDTA/mL) and sodium fluoride/potassium oxalate (2-4 mg/L sodium fluoride and 1-3 mg/L potassium oxalate; Vacuette, Greiner Bio-One International GmbH, Kremsmünster, Austria) on d 1 and 2. From d 3 on, blood was drawn from a catheter (Cavafix Certo with Splittocan, B. Braun Melsungen AG) inserted into the jugular vein; blood was collected in S-Monovette tubes containing $K_3$EDTA (1.6 mg/mL; Sarstedt AG and Co., Nümbrecht, Germany) and sodium fluoride/ potassium oxalate evacuated tubes (Greiner Bio-One International GmbH). Blood samples were placed on ice immediately after collection and subsequently centrifuged at 1,565 × g and 4°C for 20 min. The obtained plasma aliquots were stored at -20°C until analysis.

Basal plasma samples were taken for analyses of glucose, fructose, lactate, total protein, urea, triglycerides, nonesterified fatty acids (NEFA), BHB, insulin, glucagon, cortisol, growth

hormone, IGF-I, IGF binding proteins (IGFBP)-2, -3, and -4, adiponectin, and leptin before the first colostrum intake on d 1 and before feeding on d 2 to 5. On d 4, postprandial plasma concentrations of glucose, NEFA, insulin, glucagon, cortisol, and growth hormone were studied by means of hourly plasma samples taken during the first 10 h (metabolites) or 8 h (hormones) after the morning feeding. For technical reasons, basal plasma samples were not collected on d 2 to 4, and postprandial sampling was not conducted in block 2.

Metabolites were analyzed in plasma containing sodium fluoride and potassium oxalate using an automatic spectrophotometer (ABX Pentra 400; Horiba ABX) and the following kits: glucose (#A11A01667), lactate (#A11A01721), and triglycerides (#A11A01640; Horiba ABX); BHB (#RB1008) and urea (#LT-UR 0010; Labor + Technik Eberhard Lehmann GmbH); total protein (#553-412; MTI Diagnostics); and NEFA (#434-91795, #436-91995; Wako Chemicals). Fructose was analyzed in $K_3$EDTA plasma from d 1 by HPLC as previously described (Metges et al., 2014).

Concentrations of insulin and glucagon were measured in plasma containing $K_3$EDTA by RIA using corresponding kits (#RIA-1257, #RIA-1258; DRG Instruments GmbH, Marburg, Germany) adapted to bovine samples (Hammon et al., 2009). The mean intra- and interassay CV were 6.5 and 11.8% for insulin and 6.25 and 9.9% for glucagon, respectively. The revised quantitative insulin sensitivity check index (RQUICKI) was calculated according to the equation of Perseghin et al. (2001),

$$\text{RQUICKI} = 1 / [\log(\text{glucose in mg/dL}) + \log(\text{insulin in } \mu\text{U/mL}) + \log(\text{NEFA in mmol/L})],$$

to estimate insulin sensitivity as evaluated for cows by Holtenius and Holtenius (2007). Cortisol in $K_3$EDTA plasma was analyzed by ELISA as previously reported (Gruse et al., 2016). The intra- and interassay CV were 5.3 and 12.1%, respectively. Concentrations of growth hormone and IGF-I in $K_3$EDTA plasma were measured by RIA according to Vicari et al. (2008). The intra- and interassay CV for both measurements were less than 10 and 15%, respectively.

Concentrations of IGFBP-2, -3, and -4 were analyzed in $K_3$EDTA plasma by quantitative Western ligand blot analysis as described by Wirthgen et al. (2016) and Frieten et al. (2018). The intra- and interassay CV were less than 15 and 20%, respectively. Concentrations of adiponectin and leptin were determined in $K_3$EDTA plasma by ELISA; intra- and interassay CV were 7.4 and 10.9% for leptin and 9.7 and 12.7% for adiponectin, respectively (Sauerwein et al., 2004, Mielenz et al., 2013).

### *3.2.4 Determination of energy expenditure*

The rate of appearance of $CO_2$ was determined as an indirect measure of energy expenditure (Junghans et al., 2007). Thirty calves received an intravenous bolus of $NaH^{13}CO_3$ (1 mg/kg BW; 99 atom% $^{13}C$; Sigma- Aldrich) dissolved in 9 mL of saline (0.9%) directly after the morning feeding. Blood samples were collected via catheter on d 3 of life, 15 and 5 min before and 5, 7.5, 10, 15, 20, 30, 45, 60, 90, 120, 150, and 180 min after tracer application using $K_3$EDTA Monovettes (Sarstedt AG & Co). Whole blood was frozen shortly after sampling and stored at -20°C until analysis. The abundance of $^{13}C$ in blood $CO_2$ was determined according to the protocol published by Junghans et al. (2007), but 500 µL of lactic acid was used to release $CO_2$ from 500 µL of whole blood. Values for $^{13}C$ abundance in blood $CO_2$ were converted to atom percent excess and corrected for the mean $^{13}CO_2$ abundance in the blood at both time points before tracer application. Daily energy expenditure was calculated according to Junghans et al. (2007). The sum of 2 exponentials was applied to fit the kinetics of $_{13}C$ enrichment, and nonlinear regression analysis was conducted as reported by Kaufmann et al. (2011). A recovery factor of 0.81, as published by Junghans et al. (2007), was chosen to take into account incomplete $^{13}C$ recovery from blood. A respiratory quotient of 0.76 was applied based on respiration chamber measurements in 2-day-old calves during the first 3 h after milk feeding (Liermann et al., 2020).

### *3.2.5 First-pass uptake of glucose*

The first-pass uptake [i.e., the proportion of orally ingested glucose used by the intestine and liver (splanchnic tissue) when it passes the splanchnic tissue for the first time] was determined in a tracer study as described earlier (Schönhusen et al., 2013) and modified according to Gruse et al. (2015). The tracer study was conducted on d 4 and included 29 calves. Briefly, an oral bolus dose of [$^{13}C_6$]-glucose (10 mg/kg BW; 99 atom% $^{13}C$; Cambridge Isotope Laboratories, Inc.) dissolved in 9 mL of saline (0.9%) and D(+)-xylose (0.5 g/ kg BW; 99% xylose; Carl Roth GmbH + Co. KG) was mixed with 100 mL of milk and fed to the calves. After application of the oral tracer, an intravenous bolus dose of [6,6-$^2H_2$]-glucose (5 mg/kg BW; 99 atom% $^2H$; Sigma- Aldrich) dissolved in 9 mL of saline was injected via the jugular vein catheter; the catheter was then thoroughly purged with 20 mL of saline. Directly after tracer application, the residual morning meal was given. Plasma samples for analyses of [$^{13}C_6$]- and [6,6-$^2H_2$]-glucose were taken 15 and 5 min before and 5, 15, 30, 45, 60, 90, 120, 150, 180, 240, 360, 480, 600, and 1,440 min after tracer application by catheter using lithium heparinized tubes (12-13 IU heparin/mL; Vacuette; Greiner Bio-One International GmbH). Whole blood was collected for measurement of $^{13}CO_2$ enrichment in tubes containing $K_3$EDTA. The samples were taken 15 and 5 min before and 60, 120, 150, 180, 210, 240, 300, 360, 480, 600, and 1,440 min after tracer application. For xylose analysis, plasma samples were taken 15 min before and 60, 120, 180, 240, 300, 360, 420, 480, and 1,440 min after xylose application in tubes containing $K_3$EDTA. Whole blood and plasma samples were stored at -20°C until analysis. Xylose concentration in plasma was determined spectrophotometrically as previously reported (Gruse et al., 2015). The enrichment of [$^{13}C_6$]- and [6,6-$^2H_2$]- glucose in plasma was determined by GC-MS as previously described (Junghans et al., 2010). Mean natural abundances in both samples taken before application of the tracer were defined as basal tracer abundance. The rate of appearance of orally and intravenously administered glucose were determined according to the equations of Junghans et al. (2007), and from these the fractional first-pass uptake was calculated as

described by Gruse et al. (2015) based on the area under the enrichment curves (AUC; mole percent excess × min). The enrichment of $^{13}C$ in blood $CO_2$ was analyzed as a measure for glucose oxidation as previously reported (Junghans et al., 2007; Gruse et al., 2015), and the mean abundance of both samples taken before tracer application was used as the basal value.

### *3.2.6 Sampling and analyses of tissues*

Calves were slaughtered 2 h after feeding on d 5 of life, with the exception of 3 calves, which were slaughtered on d 6 for technical reasons (2 calves from group EFA+CLA and 1 calf from group CLA). The weights of the liver, kidney, pancreas, spleen, and thymus were recorded. For morphometric measurements, pieces of the duodenum, mid jejunum, and ileum were sampled, rinsed with saline (0.9%), fixed in Histofix (4% formaldehyde solution; Carl Roth GmbH + Co. KG), and stored at 4°C until analysis. Morphometric measurements of the small intestine (villus circumference, villus cut surface area, villus height, and crypt depth) were conducted as previously published by Schäff et al. (2018) based on the protocol of Zitnan et al. (2008). Ten images were taken from each segment, and 30 villi and crypts were measured from each segment. The accuracy of 30 villi was tested in previous studies; the CV for measurements in the intestine could be reduced to less than 20% if at least 30 villi and crypts were evaluated (Blättler et al., 2001).

### *3.2.7 Statistical analyses*

Statistical analyses were performed using SAS for Windows (version 9.4; SAS Institute Inc.) using the MIXED procedure. The applied model included the EFA (yes, no) and CLA (yes, no) treatments, time (d relative to calving or min after feeding), block (1 to 5), sex, and their respective interactions (EFA × CLA; EFA × time; CLA × time; EFA × CLA × time) as fixed effects. The duration of maternal supplementation and gestation length were included as covariates. For analyses of milk compounds, the model included the treatments EFA (yes, no)

and CLA (yes, no), time (milking relative to parturition), block (1 to 5), and their respective interactions (EFA × CLA; EFA × time; CLA × time; EFA × CLA × time) as fixed effects and the duration of maternal supplementation and gestation length as covariates. Gut morphometry was analyzed using a model including the treatments EFA and CLA, gut segment (duodenum, jejunum, ileum), block, sex, and their interactions (EFA × CLA; EFA × gut segment; CLA × gut segment; EFA × CLA × gut segment) as fixed effects. The REPEATED statement was used to take into account repeated measures on the same calf. For measurements conducted only once per animal (e.g., plasma fructose; AUC for xylose, $^{13}CO_2$, [$^{13}C_6$]-glucose, and [6,6-$^{2}H_2$]-glucose enrichments; first-pass uptake; rate of appearance of orally administered glucose; rate of appearance of intravenously administered glucose; energy expenditure; and organ weights) a model including the EFA and CLA treatment, block, and sex as fixed effects and the calf as random effect was used. The Tukey-Kramer test was applied to analyze pairwise differences of least squares means (LSM). Partitioned analyses of the LSM for interactions were conducted using the SLICE statement of the MIXED procedure. Results are presented as LSM ± standard error (SE) unless otherwise stated. Effects were considered significant at $P < 0.05$. For analysis of the relationship between metabolite and hormone concentrations in maternal and calf plasma, Spearman’s rank correlation was applied using the CORR procedure of SAS. Correlations were regarded as significant at $P < 0.05$.

## 3.3 Results

### *3.3.1 Concentration in milk and daily intake of IGF-I, adiponectin, and leptin*

Findings for nutrient content in colostrum and transition milk, as well as nutrient intake, were recently published in a companion paper (Uken et al., 2021; Chapter 2, Figure 2.1 and Table 2.1). The concentrations of IGF-I, adiponectin, and leptin in milk decreased ($P < 0.001$) during early lactation in all groups (Table 3.1). The concentrations of IGF-I in colostrum and transition milk were similar among the groups. The concentrations of adiponectin tended to be affected by maternal CLA supply ($P = 0.09$), with a higher concentration in milking 1 (first colostrum) in the EFA+CLA group than in the CTRL group ($P = 0.04$) and a higher concentration in milking 2 in the CLA group than in the CTRL, EFA, and EFA+CLA groups ($P < 0.01$). The leptin concentration in milking 1 (first colostrum) was higher in the EFA group than in the CTRL group ($P = 0.03$) but remained similar afterward. The intake of IGF-I did not differ among the groups during the first 5 d of life (Table 3.1). The intake of adiponectin on d 1 was higher ($P < 0.001$) in CLA calves than in non-CLA calves (LSM ± SE for CLA = 7.66 ± 0.31 mg/kg BW and for non-CLA = 6.22 ± 0.50 mg/ kg BW) and higher ($P = 0.04$) in CLA calves than in CTRL calves. The intake of leptin on d 1 was higher in EFA and CLA calves than in CTRL calves ($P < 0.05$)

**Table 3.1: Concentration in milk (fresh matter) and daily intake of IGF-I, adiponectin, and leptin for calves whose dams were supplemented with coconut oil (CTRL; n = 9), linseed and safflower oil (EFA; n = 9), Lutalin (BASF SE; CLA; *cis*-9, *trans*-11 and *trans*-10, *cis*-12; n = 9), or EFA and CLA (EFA+CLA; n = 10)[1]**

| Item | Time[2] | Supplementation | | | | *P*-value[3] | | |
|---|---|---|---|---|---|---|---|---|
| | | CTRL | EFA | CLA | EFA+CLA | EFA<br>Time | CLA<br>EFA × Time | EFA × CLA<br>CLA × Time |
| Concentration in colostrum and transition milk | | | | | | | | |
| IGF-I | 1 | 399.7 ± 78.3 | 566.1 ± 77.5 | 407.5 ± 77.4 | 436.7 ± 73.5 | 0.52 | 0.35 | 0.79 |
| µg/kg | 2 | 318.4 ± 108.9 | 372.7 ± 107.8 | 352.3 ± 130.8 | 332.5 ± 106.4 | 0.04 | 1.00 | 1.00 |
| | 3 | 236.2 ± 64.4 | 230.2 ± 55.1 | 160.0 ± 47.4 | 183.6 ± 67.8 | | | |
| | 4 | 143.4 ± 57.5 | 146.3 ± 47.8 | 81.1 ± 67.7 | 131.3 ± 43.2 | | | |
| | 5 | 106.8 ± 36.0 | 96.0 ± 32.0 | 78.1 ± 30.8 | 77.4 ± 27.6 | | | |
| | 6 | 75.2 ± 39.0 | 58.4 ± 36.5 | 45.9 ± 29.0 | 60.8 ± 27.5 | | | |
| | 7 | 40.1 ± 40.1 | 64.1 ± 43.7 | 36.4 ± 33.3 | 52.0 ± 49.6 | | | |
| | 8 | 49.3 ± 53.5 | 31.8 ± 42.7 | 48.2 ± 84.1 | 33.9 ± 62.5 | | | |
| | 9 | 18.3 ± 59.0 | 37.3 ± 74.6 | 41.0 ± 49.8 | 38.6 ± 46.0 | | | |
| Adiponectin, | 1 | 58.59 ± 5.33$^{b}$ | 65.61 ± 5.21$^{ab}$ | 75.57 ± 5.22$^{ab}$ | 78.25 ± 4.95$^{a}$ | 0.71 | 0.09 | 0.74 |
| mg/kg | 2 | 47.48 ± 7.88$^{b}$ | 47.69 ± 7.82$^{b}$ | 85.33 ± 7.84$^{a}$ | 43.94 ± 7.83$^{b}$ | <0.001 | 0.20 | 0.25 |
| | 3 | 23.52 ± 6.49 | 22.96 ± 5.93 | 23.38 ± 5.53 | 36.28 ± 6.44 | | | |
| | 4 | 11.62 ± 7.95 | 14.56 ± 7.03 | 14.73 ± 9.00 | 20.18 ± 6.95 | | | |
| | 5 | 16.50 ± 5.63 | 9.07 ± 5.51 | 9.74 ± 5.53 | 11.61 ± 4.95 | | | |
| | 6 | 7.78 ± 6.55 | 8.12 ± 6.41 | 5.49 ± 5.56 | 14.77 ± 5.22 | | | |
| | 7 | 11.36 ± 7.12 | 5.08 ± 7.00 | 4.03 ± 5.94 | 7.05 ± 6.41 | | | |
| | 8 | 4.65 ± 9.14 | 10.45 ± 7.03 | 19.54 ± 9.01 | 2.53 ± 7.84 | | | |
| | 9 | 6.70 ± 11.18 | 2.84 ± 11.01 | 3.14 ± 7.80 | 7.58 ± 9.09 | | | |

**Table 3.1: Continuation**

| Item | Time[2] | Supplementation | | | | *P*-value[3] | | |
|---|---|---|---|---|---|---|---|---|
| | | CTRL | EFA | CLA | EFA+CLA | EFA<br>Time | CLA<br>EFA × Time | EFA × CLA<br>CLA × Time |
| Leptin, | 1 | 18.36 ± 2.67[b] | 29.06 ± 2.58[a] | 25.07 ± 2.58[ab] | 21.73 ± 2.45[ab] | 0.91 | 0.89 | 0.32 |
| µg/kg | 2 | 12.24 ± 3.72 | 18.13 ± 3.65 | 16.42 ± 3.65 | 12.17 ± 3.62 | <0.001 | 0.85 | 1.00 |
| | 3 | 9.17 ± 3.14 | 10.36 ± 2.86 | 12.90 ± 2.70 | 9.66 ± 3.05 | | | |
| | 4 | 7.08 ± 3.77 | 7.72 ± 3.33 | 9.69 ± 4.15 | 7.08 ± 3.28 | | | |
| | 5 | 6.62 ± 2.79 | 7.00 ± 2.70 | 5.76 ± 2.70 | 5.76 ± 2.45 | | | |
| | 6 | 5.93 ± 3.17 | 6.05 ± 3.07 | 4.27 ± 2.72 | 4.70 ± 2.56 | | | |
| | 7 | 3.39 ± 3.40 | 3.67 ± 3.31 | 3.87 ± 2.87 | 4.28 ± 3.04 | | | |
| | 8 | 6.24 ± 4.28 | 4.75 ± 3.33 | 6.24 ± 4.15 | 1.53 ± 3.63 | | | |
| | 9 | 2.38 ± 4.27 | 2.57 ± 5.00 | 0.94 ± 3.64 | 4.26 ± 4.17 | | | |
| Intake per kg BW during the first 5 d of life | | | | | | | | |
| IGF-I, | 1 | 41.74 ± 3.28 | 50.53 ± 3.24 | 40.40 ± 3.05 | 40.66 ± 2.77 | 0.85 | 0.17 | 0.95 |
| µg/kg BW | 2 | 18.56 ± 3.30 | 13.61 ± 3.05 | 12.87 ± 3.05 | 13.63 ± 2.77 | <0.001 | 0.42 | 0.68 |
| | 3 | 13.98 ± 3.49 | 14.50 ± 3.05 | 10.31 ± 3.05 | 11.31 ± 2.77 | | | |
| | 4 | 7.31 ± 3.48 | 6.80 ± 3.22 | 6.58 ± 3.20 | 6.79 ± 3.02 | | | |
| | 5 | 5.29 ± 3.30 | 3.81 ± 3.05 | 5.21 ± 3.05 | 4.28 ± 2.77 | | | |
| Adiponectin, | 1 | 6.22 ± 0.50[b] | 6.61 ± 0.49[ab] | 8.04 ± 0.46[a] | 7.27 ± 0.42[ab] | 0.70 | 0.20 | 0.50 |
| mg/kg BW | 2 | 1.85 ± 0.50 | 1.39 ± 0.46 | 2.48 ± 0.46 | 2.32 ± 0.42 | <0.001 | 0.93 | 0.11 |
| | 3 | 2.14 ± 0.50 | 1.31 ± 0.46 | 1.40 ± 0.46 | 1.64 ± 0.42 | | | |
| | 4 | 1.24 ± 0.53 | 0.76 ± 0.49 | 0.60 ± 0.49 | 1.42 ± 0.46 | | | |
| | 5 | 0.72 ± 0.53 | 0.75 ± 0.46 | 0.35 ± 0.49 | 0.57 ± 0.42 | | | |

**Table 3.1: Continuation**

| Item | Time[2] | Supplementation | | | | *P*-value[3] | | |
|---|---|---|---|---|---|---|---|---|
| | | CTRL | EFA | CLA | EFA+CLA | EFA<br>Time | CLA<br>EFA × Time | EFA × CLA<br>CLA × Time |
| Leptin, | 1 | 1.49 ± 0.26[b] | 2.48 ± 0.25[a] | 2.54 ± 0.24[a] | 1.85 ± 0.22[ab] | 0.91 | 0.74 | 0.29 |
| µg/kg BW | 2 | 0.62 ± 0.26 | 0.59 ± 0.24 | 0.81 ± 0.24 | 0.75 ± 0.22 | <0.001 | 0.88 | 0.82 |
| | 3 | 0.84 ± 0.26 | 0.84 ± 0.24 | 0.86 ± 0.24 | 0.69 ± 0.22 | | | |
| | 4 | 0.54 ± 0.27 | 0.57 ± 0.25 | 0.61 ± 0.25 | 0.52 ± 0.23 | | | |
| | 5 | 0.51 ± 0.27 | 0.41 ± 0.24 | 0.48 ± 0.24 | 0.39 ± 0.22 | | | |

[a,b]LSM values within a row with different lowercase letters differed between treatments ($P < 0.05$).

[1]Values are presented as LSM ± SE.

[2]Time was either milking number relative to parturition (2 times milking per day) for concentrations of hormones in milk, or day of life for intake of hormones.

[3]*P*-values for fixed effects are presented in 2 rows: The first row indicates P-values for the effect of EFA, CLA, and their interaction; the second row indicates P-values for milking or day of life and interactions between EFA or CLA and milking or day of life.

### *3.3.2 Metabolites and hormones in basal blood samples*

Plasma glucose concentrations directly after birth and before first colostrum intake were higher in EFA calves than in CTRL and CLA calves ($P < 0.05$; Table 3.2). Plasma glucose on d 1 was higher ($P < 0.05$) in EFA calves than in non-EFA calves (LSM ± SE for EFA = 5.62 ± 0.43 mmol/L and for non-EFA = 4.03 ± 0.49 mmol/L), and was lower ($P < 0.05$) in CLA calves than in non-CLA calves (LSM ± SE for CLA = 4.38 ± 0.44 mmol/L and for non-CLA = 5.27 ± 0.48 mmol/L). The basal plasma glucose increased distinctly ($P < 0.05$) from d 1 to d 2 of life in non-EFA calves and remained unchanged until d 5 of life. Plasma fructose was measurable only on d 1 of life and did not differ among the groups.

Plasma lactate concentrations decreased ($P < 0.001$) from d 1 to d 2 in all groups but indicated no treatment effects. Plasma BHB concentrations were highest on d 3 of life in all calves, and maternal CLA supplementation tended to affect BHB ($P = 0.08$), with lower concentrations in the CLA group than in the CTRL group on d 4 ($P < 0.01$). Plasma concentrations of total protein increased ($P < 0.001$) from d 1 to d 2 in all groups and were higher on d 2 ($P = 0.01$) in CLA calves than in non-CLA calves. Plasma urea concentrations increased ($P < 0.001$) from d 1 to d 4 and decreased ($P < 0.001$) afterward up to d 5 of life in all groups. Calves in the EFA group tended to have lower urea concentrations ($P = 0.05$) than non-EFA calves on d 2 of life (LSM ± SE for EFA = 3.63 ± 0.77 mmol/L and for non-EFA = 5.21 ± 0.86 mmol/L). Plasma concentrations of triglycerides increased ($P < 0.05$) and NEFA decreased ($P < 0.001$) after birth in all groups, but neither metabolite showed treatment effects until d 5 of life.

Basal plasma insulin concentrations did not change with time and were not affected by treatment (Table 3.2). Plasma concentrations of glucagon increased ($P < 0.001$) after birth in all groups and on d 2 were lower ($P < 0.05$) in EFA calves compared with non-EFA calves (LSM ± SE for EFA = 201.3 ± 29.3 ng/L and for non- EFA = 287.2 ± 30.3 ng/L). The ratio of glucagon to insulin on d 2 was lower ($P = 0.03$) in EFA calves than in non-EFA calves (LSM

± SE for EFA = 0.82 ± 0.28 and for non-EFA = 1.59 ± 0.30). The ratio of glucose to insulin did not respond to the different treatments of the dams. However, the RQUICKI index on d 1 was higher ($P < 0.05$) in the CLA calves than in the non- CLA calves (LSM ± SE for CLA = 0.50 ± 0.03 and for non-CLA = 0.41 ± 0.03). Plasma cortisol concentrations decreased ($P < 0.05$) and plasma growth hormone concentrations increased ($P < 0.05$) after birth, but the plasma concentrations of both hormones were not affected by maternal fatty acid supplementation. Plasma IGF-I concentrations decreased after birth, were higher ($P < 0.05$) on d 1 of life in EFA calves than in EFA+CLA calves, and indicated an overall CLA effect ($P < 0.05$), with lower plasma IGF-I in CLA calves than in non- CLA calves throughout the study (LSM ± SE for CLA = 114.1 ± 14.7 µg/L and for non-CLA = 139.2 ± 15.9 µg/L). Plasma concentrations of IGFBP-3 increased from d 1 to 2 in all groups except for the CTRL group ($P < 0.01$), and higher concentrations were observed on d 2 in EFA calves than in non-EFA calves ($P = 0.04$; LSM ± SE for EFA = 2,240 ± 179 µg/L and for non-EFA = 1,868 µ 202 µg/L). Ratios of IGFBP-3 to IGFBP-2 in plasma increased ($P < 0.001$) from d 1 to d 2, and ratios were higher ($P < 0.05$) on d 2 in EFA calves than in EFA+CLA calves. Plasma IGFBP-4 concentrations increased ($P < 0.05$) from d 1 to d 2 in all groups and remained elevated until d 5 but did not show effects with respect to maternal fatty acid treatment. Plasma adiponectin concentrations increased ($P < 0.001$) from d 1 to d 2 in all groups, remained elevated throughout the study, and were higher ($P = 0.03$) on d 3 in CLA calves than in non-CLA calves (LSM ± SE for CLA = 14.4 ± 1.1 mg/L and for non-CLA = 12.0 ± 1.1 mg/L). Plasma concentrations of leptin increased from d 1 to 2 in calves from the EFA and EFA+CLA groups ($P < 0.05$) and were higher ($P < 0.05$) on d 4 and 5 in EFA calves than in non-EFA calves (LSM ± SE on d 4 for EFA = 4.82 ± 0.63 µg/L and for non-EFA = 3.49 ± 0.71 µg/L; on d 5 for EFA = 4.79 ± 0.60 µg/L and for non-EFA = 3.50 ± 0.69 µg/L)

**Table 3.2: Concentrations of metabolites and hormones from d 1 to 5 of life in the basal plasma before feeding of calves whose dams were supplemented with coconut oil (CTRL; n= 9), linseed and safflower oil (EFA; n = 9), Lutalin (BASF SE; CLA; *cis*-9, *trans*-11 and *trans*-10, *cis*-12; n = 9), or EFA and CLA (EFA+CLA; n = 11)[1]**

| | | Maternal supplementation[4] | | | | | | | | *P*-value[5] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Item[2] | Time[3] | CTRL | | EFA | | CLA | | EFA+CLA | | EFA<br>Time | CLA<br>EFA × Time | EFA × CLA<br>CLA × Time |
| Glucose, | 1 | 4.35 ± | 0.59[b] | 6.18 ± | 0.56[a] | 3.70 ± | 0.58[b] | 5.07 ± | 0.49[ab] | 0.23 | 0.43 | 0.98 |
| mmol/L | 2 | 6.27 ± | 0.63 | 5.65 ± | 0.59 | 5.23 ± | 0.59 | 5.57 ± | 0.55 | <0.001 | <0.01 | 0.44 |
| | 3 | 4.93 ± | 0.64 | 5.22 ± | 0.60 | 4.99 ± | 0.59 | 5.09 ± | 0.56 | | | |
| | 4 | 5.88 ± | 0.63 | 5.98 ± | 0.59 | 6.40 ± | 0.59 | 5.88 ± | 0.55 | | | |
| | 5 | 6.06 ± | 0.59 | 6.30 ± | 0.56 | 6.00 ± | 0.58 | 6.60 ± | 0.49 | | | |
| Fructose, mmol/L[6] | 1 | 2.71 ± | 0.42 | 2.05 ± | 0.38 | 2.23 ± | 0.40 | 2.23 ± | 0.33[7] | 0.25 | 0.58 | 0.21 |
| Lactate, | 1 | 5.94 ± | 0.82 | 5.61 ± | 0.77 | 4.81 ± | 0.80 | 4.39 ± | 0.67 | 0.93 | 0.99 | 0.68 |
| mmol/L | 2 | 2.62 ± | 0.87 | 2.96 ± | 0.82 | 3.04 ± | 0.82 | 3.32 ± | 0.76 | <0.001 | 0.89 | 0.20 |
| | 3 | 2.88 ± | 0.88 | 2.44 ± | 0.83 | 2.45 ± | 0.82 | 3.22 ± | 0.77 | | | |
| | 4 | 2.25 ± | 0.87 | 2.41 ± | 0.82 | 2.63 ± | 0.82 | 2.88 ± | 0.76 | | | |
| | 5 | 2.55 ± | 0.82 | 2.15 ± | 0.77 | 2.49 ± | 0.80 | 2.64 ± | 0.67 | | | |
| BHB, | 1 | 0.03 ± | 0.01 | 0.03 ± | 0.01 | 0.02 ± | 0.01 | 0.03 ± | 0.01 | 0.54 | 0.08 | <0.05 |
| mmol/L | 2 | 0.08 ± | 0.01 | 0.06 ± | 0.01 | 0.08 ± | 0.01 | 0.07 ± | 0.01 | <0.001 | 0.68 | 0.53 |
| | 3 | 0.13 ± | 0.01 | 0.12 ± | 0.01 | 0.10 ± | 0.01 | 0.10 ± | 0.01 | | | |
| | 4 | 0.09 ± | 0.01[a] | 0.06 ± | 0.01[ab] | 0.04 ± | 0.01[b] | 0.08 ± | 0.01[ab] | | | |
| | 5 | 0.07 ± | 0.01 | 0.06 ± | 0.01 | 0.05 ± | 0.01 | 0.06 ± | 0.01 | | | |

**Table 3.2: Continuation**

| Item[2] | Time[3] | Maternal supplementation[4] | | | | *P*-value[5] | | |
|---|---|---|---|---|---|---|---|---|
| | | CTRL | EFA | CLA | EFA+CLA | EFA<br>Time | CLA<br>EFA × Time | EFA × CLA<br>CLA × Time |
| Total protein, g/L | 1 | 38.6 ± 3.0 | 35.0 ± 2.8 | 37.6 ± 2.9 | 37.0 ± 2.5 | 0.31<br><0.001 | 0.07<br>0.84 | 0.39<br>0.09 |
| | 2 | 59.2 ± 3.1[ab] | 52.9 ± 2.9[b] | 62.0 ± 3.0[a] | 61.6 ± 2.7[a] | | | |
| | 3 | 58.9 ± 3.2 | 56.0 ± 3.0 | 61.4 ± 3.0 | 61.6 ± 2.7 | | | |
| | 4 | 60.1 ± 3.1 | 57.3 ± 2.9 | 62.7 ± 3.0 | 62.5 ± 2.7 | | | |
| | 5 | 57.8 ± 3.0 | 56.9 ± 2.8 | 59.4 ± 2.9 | 58.4 ± 2.5 | | | |
| Urea, mmol/L | 1 | 4.00 ± 1.01 | 3.17 ± 0.95 | 3.92 ± 0.98 | 3.51 ± 0.83 | 0.54<br><0.001 | 0.94<br>0.07 | 0.32<br>0.71 |
| | 2 | 5.67 ± 1.06 | 3.46 ± 0.99 | 4.76 ± 1.00 | 3.81 ± 0.90 | | | |
| | 3 | 6.76 ± 1.07 | 5.53 ± 1.00 | 6.63 ± 1.00 | 6.83 ± 0.92 | | | |
| | 4 | 6.43 ± 1.06 | 6.34 ± 0.99 | 5.39 ± 1.00 | 7.37 ± 0.90 | | | |
| | 5 | 4.72 ± 1.01 | 4.52 ± 0.95 | 4.33 ± 0.98 | 4.46 ± 0.83 | | | |
| Triglycerides, mmol/L | 1 | 0.23 ± 0.08 | 0.15 ± 0.08 | 0.19 ± 0.08 | 0.12 ± 0.07 | 0.23<br><0.001 | 0.68<br>0.51 | 0.98<br>0.94 |
| | 2 | 0.32 ± 0.09 | 0.19 ± 0.09 | 0.28 ± 0.08 | 0.24 ± 0.08 | | | |
| | 3 | 0.50 ± 0.09 | 0.43 ± 0.09 | 0.49 ± 0.08 | 0.51 ± 0.08 | | | |
| | 4 | 0.61 ± 0.09 | 0.73 ± 0.09 | 0.62 ± 0.08 | 0.62 ± 0.08 | | | |
| | 5 | 0.59 ± 0.08 | 0.55 ± 0.08 | 0.61 ± 0.08 | 0.48 ± 0.07 | | | |
| NEFA, µmol/L | 1 | 544 ± 93 | 583 ± 90 | 597 ± 91 | 578 ± 77 | 0.87<br><0.001 | 0.89<br>0.67 | 0.89<br>0.99 |
| | 2 | 385 ± 101 | 323 ± 96 | 377 ± 94 | 287 ± 91 | | | |
| | 3 | 440 ± 101 | 447 ± 96 | 449 ± 94 | 429 ± 92 | | | |
| | 4 | 324 ± 101 | 373 ± 96 | 285 ± 94 | 416 ± 91 | | | |
| | 5 | 291 ± 93 | 263 ± 90 | 222 ± 91 | 280 ± 77 | | | |

**Table 3.2: Continuation**

| Item[2] | Time[3] | Maternal supplementation[4] CTRL | EFA | CLA | EFA+CLA | *P*-value[5] EFA | CLA | EFA × CLA | Time | EFA × Time | CLA × Time |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Insulin, | 1 | 0.29 ± 0.21 | 0.63 ± 0.21 | 0.24 ± 0.21 | 0.48 ± 0.18 | 0.25 | 0.29 | 0.73 | | | |
| µg/L | 2 | 0.58 ± 0.13 | 0.52 ± 0.12 | 0.30 ± 0.12 | 0.52 ± 0.11 | 0.13 | 0.32 | 0.69 | | | |
| | 4 | 0.37 ± 0.12 | 0.37 ± 0.11 | 0.38 ± 0.11 | 0.30 ± 0.10 | | | | | | |
| | 5 | 0.38 ± 0.11 | 0.33 ± 0.10 | 0.33 ± 0.11 | 0.36 ± 0.09 | | | | | | |
| Glucagon, | 1 | 106.3 ± 15.4 | 86.60 ± 13.6 | 97.6 ± 14.6 | 88.4 ± 11.8 | 0.43 | 0.72 | 0.51 | | | |
| ng/L | 2 | 269.5 ± 42.6 | 208.67 ± 42.1 | 304.8 ± 40.1 | 193.9 ± 38.9 | <0.001 | <0.01 | 0.96 | | | |
| | 4 | 221.6 ± 54.6 | 308.13 ± 54.2 | 278.8 ± 53.3 | 289.0 ± 50.8 | | | | | | |
| | 5 | 202.0 ± 30.5 | 180.83 ± 29.8 | 224.0 ± 30.2 | 182.1 ± 26.7 | | | | | | |
| Glucose/Insulin | 1 | 156 ± 32 | 148 ± 29 | 135 ± 31 | 142 ± 30 | 0.80 | 0.85 | 0.99 | | | |
| mmol/nmol | 2 | 126 ± 22 | 124 ± 21 | 151 ± 21 | 133 ± 20 | 0.02 | 0.91 | 0.61 | | | |
| | 4 | 160 ± 24 | 147 ± 23 | 151 ± 22 | 164 ± 21 | | | | | | |
| | 5 | 156 ± 25 | 168 ± 25 | 168 ± 25 | 157 ± 20 | | | | | | |
| Glucagon/Insulin, | 1 | 1.85 ± 1.35 | 0.65 ± 1.28 | 2.58 ± 1.28 | 2.46 ± 1.15 | 0.32 | 0.19 | 0.90 | | | |
| mol/mol | 2 | 1.20 ± 0.40 | 0.77 ± 0.38 | 1.97 ± 0.37 | 0.87 ± 0.35 | 0.49 | 0.08 | 0.43 | | | |
| | 4 | 1.17 ± 0.31 | 1.32 ± 0.30 | 1.25 ± 0.29 | 1.45 ± 0.28 | | | | | | |
| | 5 | 1.21 ± 0.25 | 1.07 ± 0.23 | 1.34 ± 0.25 | 1.09 ± 0.20 | | | | | | |
| RQUICKI | 1 | 0.43 ± 0.05 | 0.38 ± 0.05 | 0.50 ± 0.05 | 0.50 ± 0.04 | 0.82 | 0.21 | 0.37 | | | |
| | 2 | 0.38 ± 0.02 | 0.40 ± 0.02 | 0.43 ± 0.02 | 0.41 ± 0.02 | 0.08 | 0.82 | 0.08 | | | |
| | 4 | 0.42 ± 0.05 | 0.50 ± 0.05 | 0.42 ± 0.05 | 0.41 ± 0.05 | | | | | | |
| | 5 | 0.42 ± 0.02 | 0.44 ± 0.02 | 0.44 ± 0.02 | 0.42 ± 0.01 | | | | | | |

**Table 3.2: Continuation**

| Item[2] | Time[3] | Maternal supplementation[4] | | | | *P*-value[5] | | |
|---|---|---|---|---|---|---|---|---|
| | | CTRL | EFA | CLA | EFA+CLA | EFA<br>Time | CLA<br>EFA × Time | EFA × CLA<br>CLA × Time |
| Cortisol, | 1 | 72.5 ± 6.1 | 71.4 ± 5.8 | 70.0 ± 6.0 | 68.9 ± 5.05 | 0.66 | 0.52 | 0.25 |
| µg/L | 2 | 22.1 ± 6.5 | 26.4 ± 6.1 | 33.7 ± 6.1 | 21.4 ± 5.57 | <0.001 | 0.31 | 0.66 |
| | 3 | 27.7 ± 6.6 | 21.6 ± 6.1 | 31.2 ± 6.1 | 22.2 ± 5.69 | | | |
| | 4 | 15.2 ± 6.5 | 20.6 ± 6.1 | 23.3 ± 6.1 | 23.1 ± 5.57 | | | |
| | 5 | 10.6 ± 6.1 | 19.4 ± 5.8 | 19.3 ± 6.0 | 15.6 ± 5.05 | | | |
| GH, | 1 | 8.01 ± 4.58 | 14.68 ± 4.36 | 10.69 ± 4.47 | 12.33 ± 3.78 | 0.40 | 0.62 | 0.95 |
| µg/L | 2 | 14.38 ± 4.90 | 26.07 ± 4.62 | 15.44 ± 4.58 | 17.83 ± 4.30 | <0.001 | 0.19 | 0.75 |
| | 3 | 18.35 ± 4.95 | 11.81 ± 4.64 | 10.79 ± 4.59 | 11.61 ± 4.38 | | | |
| | 4 | 9.36 ± 4.90 | 6.29 ± 4.62 | 8.28 ± 4.58 | 8.50 ± 4.48 | | | |
| | 5 | 8.17 ± 4.58 | 8.68 ± 4.36 | 6.70 ± 4.47 | 12.38 ± 3.78 | | | |
| IGF-I, | 1 | 162.6 ± 21.0[ab] | 206.1 ± 19.6[a] | 179.3 ± 20.9[ab] | 136.9 ± 17.1[b] | 0.44 | <0.05 | 0.13 |
| µg/L | 2 | 142.3 ± 21.8 | 188.2 ± 20.3 | 134.3 ± 20.7 | 137.1 ± 18.2 | <0.001 | 0.37 | 0.90 |
| | 3 | 103.4 ± 22.3 | 124.8 ± 20.4 | 89.5 ± 20.8 | 98.4 ± 18.5 | | | |
| | 4 | 113.4 ± 21.8 | 127.3 ± 20.3 | 96.0 ± 21.0 | 88.9 ± 18.5 | | | |
| | 5 | 103.0 ± 21.0 | 120.7 ± 19.6 | 93.2 ± 20.4 | 87.3 ± 17.1 | | | |
| IGFBP-2 | 1 | 1050 ± 297 | 581 ± 274 | 573 ± 288 | 846 ± 238 | 0.76 | 0.99 | 0.10 |
| µg/L | 2 | 894 ± 301 | 406 ± 278 | 457 ± 289 | 747 ± 243 | 0.01 | 0.97 | 0.59 |
| | 3 | 897 ± 302 | 520 ± 279 | 620 ± 290 | 837 ± 245 | | | |
| | 4 | 971 ± 301 | 667 ± 278 | 680 ± 289 | 944 ± 243 | | | |
| | 5 | 758 ± 297 | 582 ± 274 | 705 ± 288 | 899 ± 238 | | | |

**Table 3.2: Continuation**

| Item[2] | Time[3] | Maternal supplementation[4] CTRL | EFA | CLA | EFA+CLA | *P*-value[5] EFA | CLA | EFA × CLA | Time | EFA × Time | CLA × Time |
|---|---|---|---|---|---|---|---|---|---|---|---|
| IGFBP-3 | 1 | 1383 ± 237 | 1546 ± 221 | 1312 ± 230 | 1301 ± 193 | 0.23 | 0.54 | 0.74 | | | |
| µg/L | 2 | 1856 ± 245 | 2240 ± 228 | 1880 ± 233 | 2239 ± 204 | <0.001 | 0.18 | 0.75 | | | |
| | 3 | 1231 ± 247 | 1446 ± 230 | 1058 ± 234 | 1436 ± 208 | | | | | | |
| | 4 | 1233 ± 245 | 1143 ± 228 | 985 ± 233 | 1263 ± 204 | | | | | | |
| | 5 | 1155 ± 237 | 1130 ± 221 | 979 ± 230 | 1076 ± 193 | | | | | | |
| IGFBP-4 | 1 | 302 ± 413 | 178 ± 372 | 68 ± 373 | 295 ± 315 | 0.41 | 0.23 | 0.11 | | | |
| µg/L | 2 | 1950 ± 409 | 1910 ± 376 | 1985 ± 379 | 2651 ± 339 | <0.001 | 0.61 | 0.54 | | | |
| | 3 | 1506 ± 411 | 1469 ± 379 | 1571 ± 381 | 2322 ± 346 | | | | | | |
| | 4 | 1579 ± 405 | 1476 ± 376 | 1516 ± 379 | 2181 ± 340 | | | | | | |
| | 5 | 1630 ± 387 | 1147 ± 361 | 1424 ± 373 | 1764 ± 315 | | | | | | |
| IGFBP-3/IGFBP-2 | 1 | 2.15 ± 0.59 | 2.81 ± 0.56 | 2.40 ± 0.58 | 1.80 ± 0.49 | 0.82 | 0.20 | 0.22 | | | |
| µg/µg | 2 | 3.67 ± 0.63[ab] | 4.98 ± 0.59[a] | 3.96 ± 0.59[ab] | 3.13 ± 0.54[b] | <0.001 | 0.85 | 0.84 | | | |
| | 3 | 2.38 ± 0.64 | 2.51 ± 0.60 | 2.06 ± 0.59 | 1.89 ± 0.55 | | | | | | |
| | 4 | 2.22 ± 0.63 | 1.70 ± 0.59 | 1.93 ± 0.59 | 1.59 ± 0.54 | | | | | | |
| | 5 | 2.01 ± 0.59 | 1.97 ± 0.56 | 2.01 ± 0.58 | 1.68 ± 0.49 | | | | | | |
| Adiponectin, | 1 | 2.76 ± 1.35 | 1.72 ± 1.27 | 1.84 ± 1.32 | 2.37 ± 1.11 | 0.73 | 0.18 | 0.57 | | | |
| mg/L | 2 | 11.28 ± 1.41 | 11.09 ± 1.32 | 12.42 ± 1.34 | 12.51 ± 1.19 | <0.001 | 0.72 | 0.17 | | | |
| | 3 | 12.42 ± 1.43 | 11.67 ± 1.33 | 14.11 ± 1.34 | 14.64 ± 1.22 | | | | | | |
| | 4 | 12.88 ± 1.41 | 12.58 ± 1.32 | 13.06 ± 1.34 | 15.57 ± 1.19 | | | | | | |
| | 5 | 13.62 ± 1.35 | 15.13 ± 1.27 | 14.57 ± 1.32 | 14.41 ± 1.11 | | | | | | |

**Table 3.2: Continuation**

| Item[2] | Time[3] | Maternal supplementation[4] | | | | | | | | *P*-value[5] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | CTRL | | EFA | | CLA | | EFA+CLA | | EFA | CLA | EFA × CLA |
| | | | | | | | | | | Time | EFA × Time | CLA × Time |
| Leptin, µg/L | 1 | 2.57 ± | 0.83 | 2.82 ± | 0.77 | 3.00 ± | 0.80 | 3.08 ± | 0.67 | 0.06 | 0.34 | 0.39 |
| | 2 | 3.41 ± | 0.86 | 5.27 ± | 0.80 | 4.41 ± | 0.82 | 5.03 ± | 0.72 | <0.001 | 0.16 | 0.71 |
| | 3 | 2.90 ± | 0.87 | 3.70 ± | 0.81 | 3.85 ± | 0.82 | 4.55 ± | 0.73 | | | |
| | 4 | 2.94 ± | 0.86 | 4.77 ± | 0.80 | 4.05 ± | 0.82 | 4.86 ± | 0.72 | | | |
| | 5 | 3.08 ± | 0.83 | 5.15 ± | 0.77 | 3.93 ± | 0.80 | 4.44 ± | 0.67 | | | |

[a,b]LSM values within a row with different lowercase letters differed among treatment groups ($P < 0.05$).

[1]Values are presented as the LSM ± SE.

[2]IGFBP = IGF binding protein; NEFA = nonesterified fatty acids; RQUICKI = revised quantitative insulin sensitivity check index.

[3]Day of life.

[4]Number of sampled calves from d 2–4: CTRL, n = 7; EFA, n = 7; CLA, n = 8; EFA+CLA, n = 8.

[5]*P*-values for fixed effects are presented in 2 rows: The first row indicates *P*-values for the effect of EFA, CLA, and their interaction; the second row indicates *P*-values for time and interactions between EFA or CLA and time.

[6]Fructose in plasma was only detectable on d 1 of life.

[7]Results from one calf were excluded from analyses due to technical difficulties.

Plasma concentrations of glucose in calves before first colostrum intake were positively correlated with plasma insulin (r = 0.32; $P = 0.05$) and maternal plasma glucose at parturition (r = 0.37; $P < 0.05$). Plasma concentrations of fructose in calves before first colostrum intake were positively correlated with plasma insulin (r = 0.51; $P < 0.01$) and leptin (r = 0.54; $P < 0.001$), and plasma insulin was positively correlated with plasma leptin (r = 0.36; $P < 0.05$). Plasma concentrations of IGF-I in the calves before first colostrum intake were negatively correlated with plasma lactate (r = -0.34; $P < 0.05$) and positively correlated with maternal plasma IGF-I at parturition (r = 0.36; $P < 0.05$); maternal plasma IGF-I concentrations were negatively correlated with plasma urea in calves at birth (r = -0.57; $P < 0.001$). Plasma concentrations of adiponectin in calves before first colostrum intake were negatively correlated with maternal plasma concentrations of glucose and insulin at parturition (r = -0.36 and -0.34; $P < 0.05$, respectively).

### *3.3.3 Postprandial changes in metabolites and hormones on d 4*

Plasma concentrations of glucose increased after feeding in all groups except for the CTRL group ($P < 0.05$; Figure 3.1A). Basal glucose levels were exceeded 60 min after feeding in the EFA and EFA+CLA groups and 120 min after feeding in the CLA group ($P < 0.05$). Plasma concentrations of lactate were lower in the EFA group than in the EFA+CLA group at 600 min after feeding ($P < 0.05$; Supplemental Figure S3.1A). Plasma NEFA concentrations decreased ($P < 0.05$) after feeding in all groups except the CTRL group (Figure 3.1B). Plasma NEFA concentrations were lower ($P < 0.05$) in the EFA group than in the CTRL group at 120 and 300 min after feeding and were lower ($P < 0.05$) in the CLA group than the CTRL group at 240 min after feeding. Plasma NEFA concentrations were higher ($P < 0.05$) at 60 min after feeding in CLA calves than in non-CLA calves. Postprandial plasma concentrations of triglycerides and urea resembled basal levels and were similar between groups (Supplemental Figure S3.1B, C).

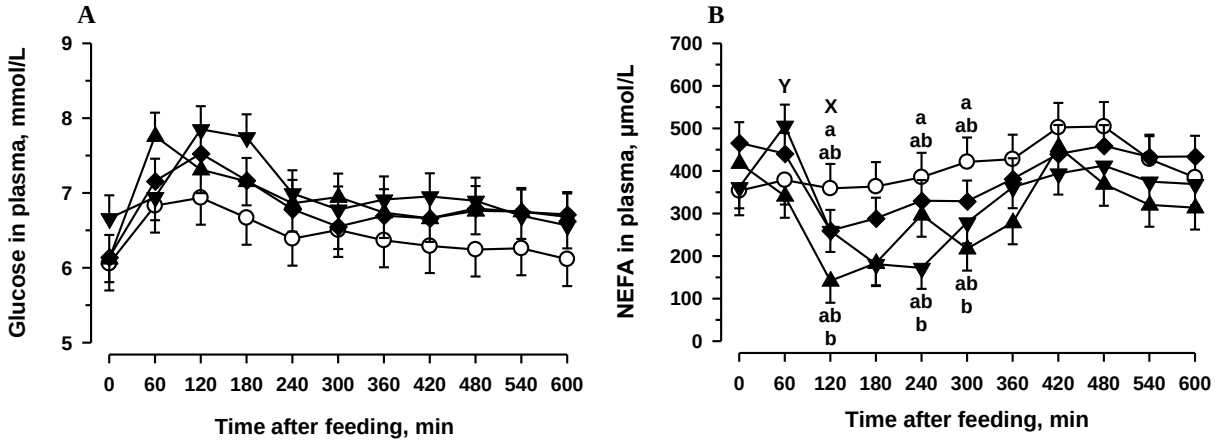


**Figure 3.1: Postprandial concentrations of (A) glucose and (B) nonesterified fatty acids (NEFA) on d 4 of life in the plasma of calves whose dams were supplemented with coconut oil (CTRL; ○; n = 6; 1 calf was excluded due to fever), linseed and safflower oil (EFA; ▲; n = 7), Lutalin (BASF SE; CLA; *cis*-9, *trans*-11 and *trans*-10, *cis*-12; ▼; n = 8), or EFA and CLA (EFA+CLA; ♦; n = 8). Data are presented as LSM and SE. Different letters (a, b) represent significant differences among groups at the same time point ($P < 0.05$). X indicates significant differences between EFA and non-EFA calves; Y indicates significant differences between CLA and non-CLA calves. Significant effects ($P < 0.05$) for glucose (time, EFA × time, and CLA × time interactions) and NEFA (EFA × CLA interaction and time).**

Plasma insulin concentrations increased ($P < 0.01$) after feeding in all groups (Figure 3.2A). Plasma insulin was higher ($P < 0.05$) 60 min after feeding in the EFA group than in the CLA group and 120 min after feeding in the EFA+CLA group than in the CTRL group. Plasma concentrations of glucagon decreased only in the calves of the EFA group 60 min after feeding ($P < 0.01$; Figure 3.2B). Ratios of glucose to insulin and glucagon to insulin decreased ($P < 0.001$) immediately after feeding, and the ratio of glucose to insulin was lower ($P < 0.01$) in EFA calves than in non-EFA calves at 420 min after feeding. The ratio of glucagon to insulin was higher ($P < 0.05$) at 360 min in CLA calves than in non-CLA calves, and it was lower (*P*

< 0.05) at 420 min after feeding in EFA calves than in non-EFA calves (Supplemental Figure S3.2A, B).

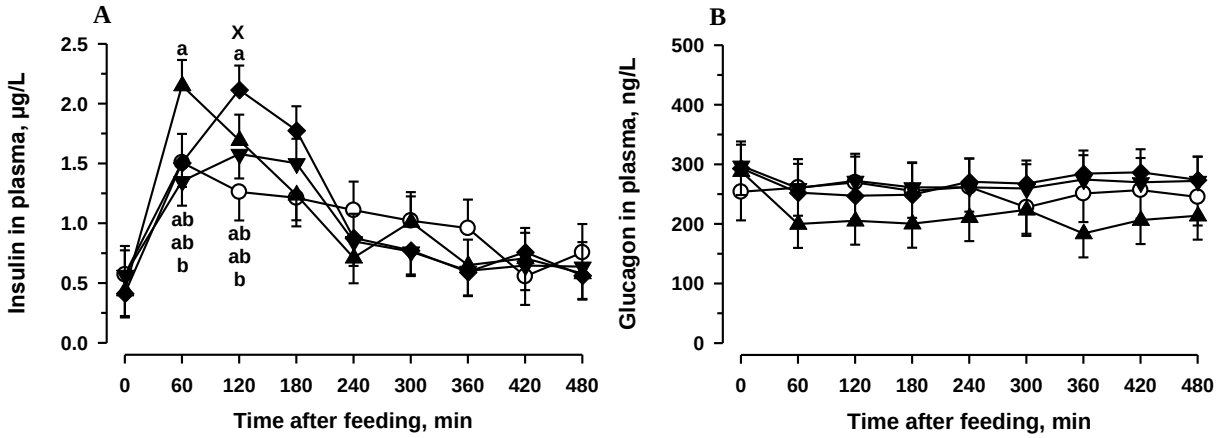


**Figure 3.2: Postprandial concentrations of (A) insulin and (B) glucagon on d 4 of life in the plasma of calves whose dams were supplemented with coconut oil (CTRL; ○; n = 6; 1 calf was excluded due to fever), linseed and safflower oil (EFA; ▲; n = 7), Lutalin (BASF SE; CLA; *cis*-9, *trans*-11 and *trans*-10, *cis*-12; ▼; n = 8), or EFA and CLA (EFA+CLA; ♦; n = 8). Data are presented as LSM and SE. Different letters (a, b) represent significant differences among groups at the same time point (*P* < 0.05). X indicates significant differences between EFA and non-EFA calves (*P* < 0.05). Significant effects (*P* < 0.05) for insulin (time and CLA × time interaction) and glucagon (time).**

Plasma cortisol concentrations decreased ($P < 0.05$) after feeding in all groups except the CTRL group and were higher ($P < 0.05$) at 300 min after feeding in the EFA group than in the CTRL group (Supplemental Figure S3.2C). Postprandial concentrations of growth hormone in plasma remained unchanged, but plasma growth hormone was higher ($P < 0.05$) at 60 and 120 min in EFA calves than in non-EFA calves (Supplemental Figure S3.3A). At 60 min after feeding, plasma growth hormone was higher in the EFA+CLA group than in the CTRL group ($P = 0.04$), and after 240 min, it was higher in the CLA group than in the EFA+CLA and CTRL

groups ($P < 0.05$). Compared with the basal values, plasma adiponectin or leptin concentrations did not show postprandial changes (Supplemental Figure S3.3B, C). However, plasma adiponectin indicated an overall CLA effect, with higher concentrations at 60, 120, 240, and 300 min after feeding in CLA calves than in non-CLA calves.

### *3.3.4 Energy expenditure and first-pass uptake of glucose*

Energy expenditure on d 3 was (LSM ± SE) 803 ± 93, 910 ± 77, 775 ± 78, and 805 ± 74 kJ/d × kg $BW^{0.75}$ in the CTRL, EFA, CLA, and EFA+CLA calves, respectively, but did not differ among groups. Plasma concentrations of xylose increased in all groups after feeding and were lower in EFA calves than in non-EFA calves at 360 and 420 min after oral xylose administration ($P < 0.05$; Figure 3.3A). Nevertheless, the AUC of plasma xylose concentration was similar among the groups (LSM ± SE; 1,866 ± 80, 1,661 ± 64, 1,669 ± 66, and 1,704 ± 61 mmol/L × min for the CTRL, EFA, CLA, and EFA+CLA groups, respectively). Enriched [$^{13}C_6$]-glucose levels exceeded basal values after feeding in all groups ($P < 0.05$; Figure 3.3B). Moreover, [$^{13}C_6$]- glucose enrichment at 15 and 30 min after feeding was higher in EFA calves than in non-EFA calves ($P < 0.05$), and [$^{13}C_6$]-glucose enrichment at 5 and 15 min after feeding was higher ($P < 0.05$) in the EFA+CLA group than in the CLA group. Nevertheless, the rate of appearance of orally administered glucose, the rate of appearance of intravenously administered glucose, and first-pass uptake with or without correction for xylose absorption were similar among groups (Table 3.3). Enrichment of $^{13}CO_2$ in blood at 360 min after feeding tended to be lower in EFA calves than in non-EFA calves ($P = 0.06$; Figure 3.3C), whereas the AUC for $^{13}CO_2$ was similar irrespective of maternal treatment.

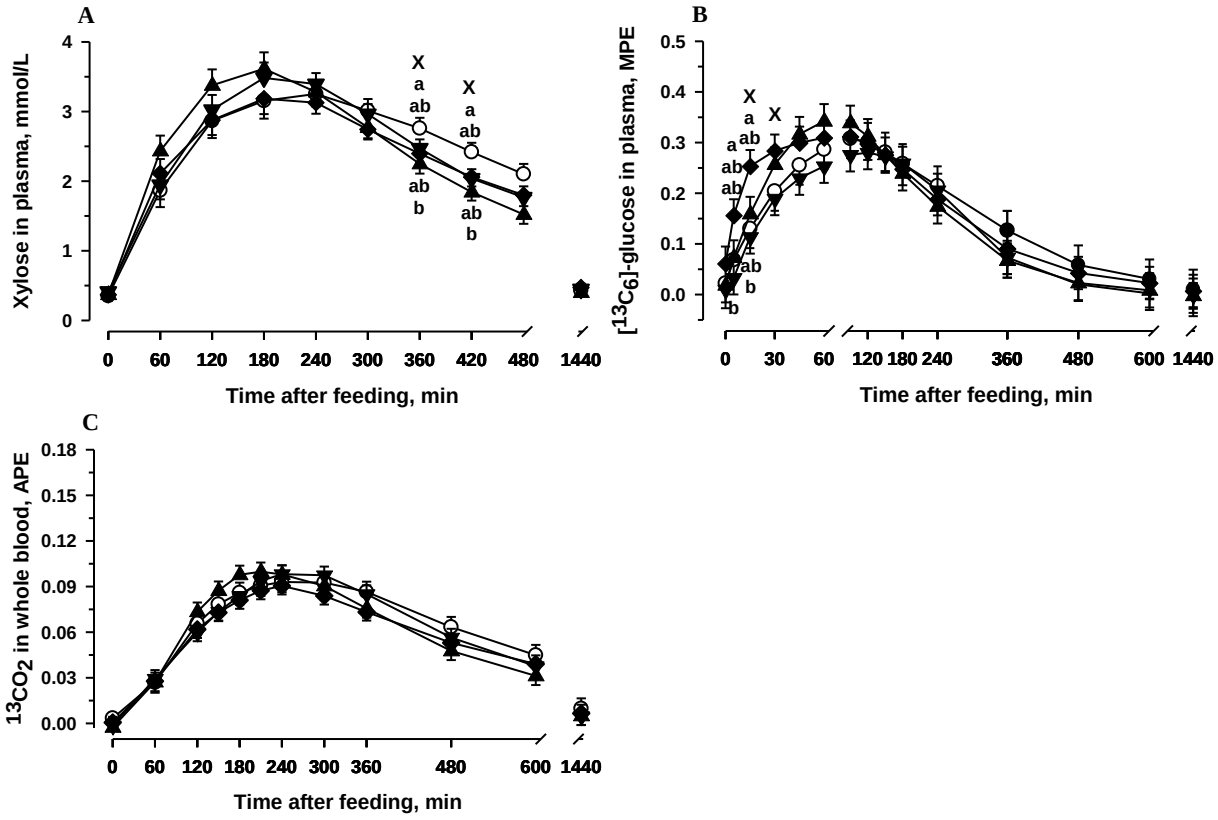


**Figure 3.3: (A) Postprandial concentrations of xylose in plasma, (B) enrichment of [$^{13}C_6$]-glucose in plasma, and (C) enrichment of $^{13}CO_2$ in blood on d 4 of life in calves whose dams were supplemented with coconut oil (CTRL; ○; n = 6; 1 calf was excluded due to fever), linseed and safflower oil (EFA; ▲; n = 7), Lutalin (BASF SE; CLA; *cis*-9, *trans*-11 and *trans*-10, *cis*-12; ▼; n = 8; 1 calf was excluded from statistical analyses of [$^{13}C_6$]-glucose due to technical difficulties), or EFA and CLA (EFA+CLA; ♦; n = 8). Data are presented as LSM and SE. Different letters (a, b) represent significant differences among groups at the same time point ($P < 0.05$). X indicates significant differences between EFA and non-EFA calves ($P < 0.05$). Significant effects ($P < 0.05$) for xylose (time), enrichment of [$^{13}C_6$]-glucose (time and EFA × time interaction), and $^{13}CO_2$ (time). MPE = mole percent excess; APE= atom percent excess.**

**Table 3.3: Area under the curve for [$^{13}C_6$]-glucose and $^{13}CO_2$ enrichment, rates of appearance, and first-pass glucose uptake of calves whose dams were supplemented with coconut oil (CTRL; n = 6), linseed and safflower oil (EFA; n = 7), Lutalin (BASF SE; CLA; *cis*-9, *trans*-11 and *trans*-10, *cis*-12; n = 8), or EFA and CLA (EFA+CLA; n = 8)[1,2]**

| | Maternal supplementation | | | | *P*-value | | |
|---|---|---|---|---|---|---|---|
| Item[3] | CTRL | EFA | CLA | EFA+CLA | EFA | CLA | EFA × CLA |
| [$^{13}C_6$]-glucose enrichment AUC[4], MPE × min | 101.0 ± 12.1 | 91.6 ± 9.6 | 93.5 ± 10.5 | 94.7 ± 9.3 | 0.71 | 0.83 | 0.61 |
| $Ra_{oral}$[4], mmol/(kg × h)[4] | 2.04 ± 1.75 | 5.77 ± 1.38 | 4.97 ± 1.52 | 5.90 ± 1.34 | 0.16 | 0.31 | 0.36 |
| $Ra_{i.v.}$, mmol/(kg × h) | 1.01 ± 0.23 | 1.32 ± 0.18 | 0.91 ± 0.20 | 0.97 ± 0.17 | 0.36 | 0.24 | 0.53 |
| Fractional FPU, %[5] | 64.7 ± 9.5 | 60.3 ± 7.5 | 76.0 ± 8.2 | 70.8 ± 7.3 | 0.58 | 0.19 | 0.97 |
| $^{13}CO_2$ enrichment AUC, APE × min | 58.5 ± 2.4 | 55.4 ± 1.9 | 57.5 ± 1.9 | 54.5 ± 1.8 | 0.16 | 0.62 | 0.97 |
| Corrected for xylose absorption | | | | | | | |
| [$^{13}C_6$]-glucose enrichment AUC[4], MPE × min[4] | 82.9 ± 9.5 | 67.1 ± 7.5 | 70.2 ± 8.2 | 70.1 ± 7.3 | 0.36 | 0.55 | 0.34 |
| $Ra_{oral}$[4], mmol/(kg × h)[4] | 1.74 ± 1.35 | 4.28 ± 1.07 | 3.70 ± 1.17 | 4.57 ± 1.04 | 0.18 | 0.33 | 0.48 |
| Fractional FPU, %[5] | 61.4 ± 13.1 | 57.2 ± 10.3 | 65.6 ± 9.9 | 63.2 ± 8.7 | 0.79 | 0.61 | 0.93 |

[1]Values are presented as the LSM ± SE.

[2]One calf (CTRL group) was excluded from the study due to fever.

[3]APE = atom percent excess; AUC = area under the curve; MPE = mole percent excess; RA = rate of appearance.

[4] One calf (CLA group) was excluded from statistical analyses due to technical difficulties.

[5]Three calves (CTRL, EFA, CLA groups) were excluded from statistical analyses due to technical difficulties.

### *3.3.5 Organ development*

The weights of the liver, kidney, pancreas, spleen, and thymus were similar among groups (Supplemental Table S3.1). Maternal fatty acid supplementation did not affect the villus size of the small intestine (Table 3.4). However, crypt depth in the ileum was lower in CLA calves than in non-CLA calves ($P = 0.03$; LSM ± SE for CLA = 249 ± 3.5 µm and for non-CLA = 255 ± 3.8 µm). Consequently, the ileal ratio of villus height to crypt depth was higher in CLA calves than in non-CLA calves ($P = 0.04$; LSM ± SE for CLA = 2.25 ± 0.06 and for non-CLA = 2.14 ± 0.06).

**Table 3.4: Morphometry of the duodenum, mid jejunum, and ileum of calves whose dams were supplemented with coconut oil (CTRL; n = 9), linseed and safflower oil (EFA; n =9), Lutalin (BASF SE; CLA; *cis*-9, *trans*-11 and *trans*-10, *cis*-12; n = 9), or EFA and CLA (EFA+CLA; n = 11)[1]**

| | Maternal supplementation | | | | *P*-value[2] | | |
|---|---|---|---|---|---|---|---|
| | | | | | EFA | CLA | EFA × CLA |
| Item | CTRL | EFA | CLA | EFA+CLA | Segment | EFA × Segment | CLA × Segment |
| Villus circumference, µm | | | | | | | |
| Duodenum | 1,334 ± 24 | 1,339 ± 22 | 1,341 ± 23 | 1,352 ± 19 | 0.64 | 0.38 | 0.97 |
| Mid jejunum | 1,305 ± 22 | 1,315 ± 20 | 1,316 ± 21 | 1,327 ± 17 | <0.001 | 0.21 | 0.45 |
| Ileum | 1,273 ± 22 | 1,277 ± 20 | 1,291 ± 21 | 1,291 ± 17 | | | |
| Villus cut surface, µm$^2$ | | | | | | | |
| Duodenum | 71,038 ±1,406 | 71,141 ±1,303 | 71,265 ±1,364 | 72,018 ±1,132 | 0.65 | 0.39 | 0.89 |
| Mid jejunum | 68,344 ±1,305 | 68,968 ±1,194 | 69,088 ±1,260 | 69,741 ±1,028 | <0.001 | 0.38 | 0.42 |
| Ileum | 67,042 ±1,329 | 67,198 ±1,220 | 67,976 ±1,285 | 68,148 ±1,053 | | | |
| Villus height, µm | | | | | | | |
| Duodenum | 592 ± 12 | 593 ± 11 | 594 ± 11 | 600 ± 10 | 0.67 | 0.38 | 0.90 |
| Mid jejunum | 574 ± 11 | 579 ± 10 | 581 ± 11 | 586 ± 9 | <0.001 | 0.39 | 0.41 |
| Ileum | 559 ± 11 | 560 ± 10 | 566 ± 11 | 568 ± 9 | | | |

**Table 3.4: Continuation**

| Item | Maternal supplementation | | | | | | | | | | | | *P*-value[2] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | CTRL | | | EFA | | | CLA | | | EFA+CLA | | | EFA | CLA | EFA × CLA |
| | | | | | | | | | | | | | Segment | EFA × Segment | CLA × Segment |
| Crypt depth, µm | | | | | | | | | | | | | | | |
| Duodenum | 249 | ± | 5 | 244 | ± | 4 | 244 | ± | 4 | 241 | ± | 4 | 0.16 | 0.12 | 0.86 |
| Mid jejunum | 235 | ± | 5 | 229 | ± | 5 | 231 | ± | 5 | 225 | ± | 4 | <0.001 | 0.72 | 0.32 |
| Ileum | 258 | ± | 5 | 253 | ± | 4 | 250 | ± | 4 | 247 | ± | 4 | | | |
| Villus height/crypt depth µm/µm | | | | | | | | | | | | | 0.43 | 0.13 | 0.91 |
| Duodenum | 2.35 | ± | 0.08 | 2.38 | ± | 0.07 | 2.40 | ± | 0.08 | 2.45 | ± | 0.06 | <0.001 | 0.36 | 0.23 |
| Mid jejunum | 2.43 | ± | 0.09 | 2.49 | ± | 0.08 | 2.50 | ± | 0.08 | 2.57 | ± | 0.07 | | | |
| Ileum | 2.13 | ± | 0.08 | 2.16 | ± | 0.07 | 2.23 | ± | 0.07 | 2.26 | ± | 0.06 | | | |

[1]Values are presented as the LSM ± SE.

[2]*P*- values for fixed effects are presented in 2 rows: The first row indicates *P*-values for the effect of EFA, CLA, and their interaction; the second row indicates *P*-values for segment and interactions between EFA or CLA and segment.

## 3.4 Discussion

### *3.4.1 Metabolic and endocrine changes at birth*

In a companion paper, we recently showed that changing the maternal supply of EFA and CLA during late gestation and early lactation affected plasma fatty acid concentrations in calves around birth (Uken et al., 2021; Chapter 2). Elevated maternal and fetal n-3 fatty acid status resulted in increased plasma glucose concentrations in calves immediately after birth. Because fetal endogenous glucose production is low and calves are often born in a hypoglycemic state, maternal EFA treatment might have improved neonatal glucose status at birth (Fowden et al., 2009; Hammon et al., 2012). Although plasma glucose concentration immediately after birth was positively correlated with maternal plasma glucose, maternal plasma glucose concentration was not affected by fatty acid treatments at parturition (Vogel et al., 2021). We speculate that maternal EFA treatment may have facilitated placental glucose transport during late pregnancy. However, we did not investigate placental glucose transport in the present study. In the literature, a detrimental effect of maternal PUFA administration on placental glucose transport was described in rats (Shrestha et al., 2020). In contrast, the prevalence of placental glucose transporters increased with gestational age in sheep (Ehrhardt and Bell, 1997), and the gestation length tended to be increased in dams receiving EFA compared to dams without EFA supplementation (Uken et al., 2021). Additional studies are needed to investigate the effects of maternal EFA supplementation on placental glucose metabolism in dairy cows. The disappearance of fructose in blood plasma after birth in the present study supports previous findings of elevated plasma fructose in bovine fetuses that was largely metabolized after birth (Kurz and Willett 1992; Tyler and Ramsey, 1993). However, in our study maternal EFA and CLA supplementation did not affect plasma fructose levels in the calves at birth.

Although we found no effect of maternal fatty acid treatment on birth weight, maternal EFA supplementation alone during late gestation resulted in increased plasma IGF-I concentrations in the calves at birth. The somatotropic axis does not control fetal growth, but IGF-I is an

important growth factor that stimulates growth and organ development in the fetus (Breier et al., 2000; Gluckman and Pinal, 2003). Because glucose is a main driver of fetal IGF-I (Gluckman and Pinal, 2003), the elevated plasma glucose concentrations we found in EFA calves could have been responsible for the higher plasma IGF-I concentrations in those calves. However, CLA treatment in our study indicated an inhibition of the EFA effect on plasma IGF-I. Previous studies in growing rats indicated that CLA might reduce plasma IGF-I levels, and that the combination of n-3 fatty acid and CLA supplementation could reduce concentrations of IGFBP-3, which probably decreased IGF-I binding capacity in blood plasma (Li et al., 1999). Plasma IGFBP-3 might also affect IGF-I levels in neonatal calves, but IGFBP-3 concentrations did not differ immediately after birth among groups in the present study. Furthermore, fetal plasma glucose stimulates plasma insulin (Oliver et al., 1996), and our data indicated a positive relationship between plasma concentrations of glucose and insulin in calves at term. However, we found no treatment effect of maternal EFA supplementation on plasma insulin in the calves immediately after birth. Interestingly, we found a significant overall negative correlation between maternal plasma IGF-I and plasma urea in calves at birth. Decreased plasma urea in calves might indicate reduced amino acid degradation and elevated fetal protein synthesis. We could not determine from this study whether maternal IGF-I affects fetal protein metabolism, but studies in sheep showed an effect of maternal nutritional status on insulin and IGF-I-induced protein synthesis in the fetus (Shen et al., 2005).

Plasma glucose concentrations immediately after birth were reduced and the RQUICKI was increased by maternal CLA treatment, indicating improved insulin sensitivity in calves when dams received CLA during late gestation (Holtenius and Holtenius, 2007). However, this result must be confirmed in further studies, because the effect of CLA on insulin sensitivity is not clear and depends on the specific CLA isomer (Taylor and Zahradka, 2004). Previous studies have indicated no increased insulin sensitivity (RQUICKI) with CLA treatment in cattle (Singh

et al., 2014) but elevated insulin resistance in rats and humans (Risérus et al., 2002; Bezan, et al., 2018).

### *3.4.2 Metabolic and endocrine changes caused by colostrum and transition milk intake*

First colostrum milking contained high concentrations of IGF-I, leptin, and adiponectin, and all of those decreased with the onset of lactation, in accordance with previous findings (Blum and Hammon, 2000; Blum and Baumrucker, 2008; Kesser et al., 2015; Palin et al., 2017). Previous studies in cattle indicated no intestinal absorption of colostral IGF-I and insulin (Grütter and Blum, 1991; Vacher et al., 1995; Hammon and Blum, 1997), but an absorption of colostral IGFBP-4 was recently discussed in neonatal calves, and IGFBP-4 is enriched in bovine colostrum (Blum and Baumrucker, 2008; Meyer et al., 2017; Liermann et al., 2020). In addition, leptin and adiponectin were absorbed from colostrum in neonatal calves (Kesser et al., 2015, 2017; Liermann et al., 2020), and leptin was absorbed from colostrum in neonatal pigs (Palin et al., 2017). The relevance of the different behaviors of colostral endocrine factors for the maturation of newborn calves is not clear and is the subject of further investigation. Adiponectin and leptin from colostrum may affect intestinal development (Palin et al., 2017) and insulin sensitivity and responses in neonates (Havel, 2002; Palou et al., 2018; Liermann et al., 2020). In the present study, maternal fatty acid supplementation seemed to have no effect on the absorption of colostral endocrine factors, although we found slight stimulatory effects of EFA and CLA treatment on leptin and adiponectin concentrations in the first colostrum after parturition.

Nutrient intake with colostrum and transition milk feeding has been described in detail in a companion paper (Uken et al., 2021). Plasma total protein increased in all groups to more than 52 g/L, indicating sufficient immunoglobulin supply in all groups (Atkinson et al., 2017). The elevated plasma total protein concentration on d 2 of life in CLA calves was not a result of enhanced IgG absorption, because plasma IgG concentrations on d 2 of life did not differ among

the calves (K. L. Uken, E. Trevisi, and H. M. Hammon, unpublished observations). In contrast, EFA calves with low plasma total protein concentrations showed corresponding low plasma urea concentrations on d 2 of life. Because protein intake and IgG absorption were not different among groups but plasma IGF-I was elevated in EFA calves, this finding might indicate enhanced protein accretion in the EFA calves, because IGF-I stimulates protein synthesis in cattle (Breier et al., 2000; Hammon et al., 2012).

Basal plasma NEFA decreased and plasma triglycerides increased during the first days of life but did not indicate treatment effects of maternal fatty acid supplementation, although fat content in the colostrum on d 1 was highest in the CTRL dams. The slight increase in plasma NEFA from d 2 to d 3 of life in all groups was probably due to the low feed intake on d 2 to equalize the milk quantities between individual calves and adjust the milk feeding to 2 meals per d in the morning and evening from d 3 onward (Uken et al., 2021).

Changes in the basal plasma concentrations of glucose, lactate, and BHB during the first 5 d of life corresponded to the findings of previous studies in calves (Hammon and Blum, 1998; Frieten et al., 2017). The lower plasma BHB concentrations on d 4 in the CLA calves is hard to explain because BHB concentrations are generally very low in neonatal calves without a functional rumen (Frieten et al., 2017); these findings might indicate lower ketone body production in the livers of CLA calves. In contrast, the higher BHB concentrations in CTRL calves than in CLA calves on d 4 could be the result of increased oxidation of medium chain fatty acids from the coconut oil supplement given to the CTRL dams (Sato, 1994; Garcia et al., 2014). Although we found no effect on plasma glucose on d 2, the lower plasma glucagon concentrations and ratio of glucagon to insulin in EFA calves might have induced lower endogenous glucose production in those calves. Glucagon stimulates endogenous glucose production in calves (Hammon et al., 2012). A possible greater absorption of glucose from colostrum, as discussed below and shown in previous studies (Steinhoff-Wagner et al., 2011;

Gruse et al., 2015), may have reduced the need for endogenous glucose production in the EFA calves.

Postprandial plasma glucose concentrations and glucose turnover, as well as first-pass uptake in splanchnic tissue, were not affected by maternal fatty acid supplementation at d 4 of life. The first-pass uptake of glucose decreased during the first week of life in neonatal calves, and our findings were within the previously published ranges (Steinhoff-Wagner et al., 2011; Gruse et al., 2015). The oral tracer study indicated faster [$^{13}C_6$]-glucose absorption during the first 30 min after feed intake in EFA calves. Interestingly, plasma glucose also reached its postprandial peak earlier in the EFA group than in all other groups. We can only speculate that maternal EFA supplementation may have influenced intestinal glucose absorption in calves. Previous findings in pigs indicated elevated intestinal glucose absorption when sows were supplemented with n-3 fatty acids (Gabler et al., 2007). An EFA deficiency resulted in impaired intestinal lactose digestion in mice (Lukovac et al., 2008). Furthermore, increased intake of n-3 fatty acids instead of monounsaturated or saturated fatty acids accelerated gastric emptying in women (Robertson et al., 2002). Thus, we cannot exclude the possibility that gastric emptying in the EFA calves was faster, allowing earlier absorption of the ingested glucose and higher plasma glucose concentrations shortly after feeding.

Although lactose intake (Uken et al., 2021; Chapter 2 Table 2.1) and postprandial plasma glucose levels did not differ among groups on d 4, the postprandial peak of plasma insulin was highest in the EFA and EFA+CLA calves. Because the ratio of glucose to insulin in plasma did not differ immediately after milk intake among the groups on d 4 (Supplemental Figure S3.2A), the greater insulin rise in plasma in the EFA and EFA+CLA calves might have been the consequence of elevated glucose absorption shortly after feeding. Plasma xylose, which is absorbed by the same intestinal route as glucose (Scharrer and Grenacher, 2000), did not differ among the groups at 60 and 120 min after milk intake. Morphometric measurements in the small intestine did not indicate differences in small intestinal absorptive capacity as a result of

maternal fatty acid supplementation. The elevated ratio of villus height to crypt cell in the ileum of CLA calves might indicate enhanced intestinal maturation in the ileum due to maternal CLA treatment but was not related to intestinal glucose absorption in calves (Blättler et al., 2001; Steinhoff-Wagner et al., 2014). The decrease in plasma xylose after 360 min was faster in EFA calves than in CTRL calves. The xylose decrease in blood plasma might have started earlier in EFA calves because of faster xylose absorption, but plasma xylose also may have indicated an effect of maternal EFA supplementation on the xylose clearance rate in calf plasma.

Whole-body energy expenditure in calves as measured by $NaH^{13}CO_3$ was not affected by maternal fatty acid supplementation. With respect to postprandial energy metabolism, plasma NEFA levels did not decrease after feeding in CTRL calves on d 4, whereas the lowest postprandial NEFA concentrations were observed in EFA and CLA calves. Because NEFA levels indicate mobilization of body fat and NEFA release is inhibited by insulin (Hadorn et al., 1997; Kühne et al., 2000), the present finding corresponded to the elevated plasma insulin levels found in EFA calves. Maternal EFA supplementation also increased neonatal plasma leptin on d 4 and 5 of life. Leptin may stimulate insulin sensitivity in calves after feeding, because leptin is known for its insulin-sensitizing effects (Palou et al., 2018; Liermann et al., 2020). In addition, n-3 fatty acid supplementation improves insulin sensitivity in mice and in cattle (Pires et al., 2008; Fortin et al., 2010; Fan et al., 2020). Furthermore, postprandial plasma NEFA levels were temporarily lower in CLA calves than in CTRL calves, despite similar postprandial insulin levels between the 2 groups. This finding was surprising because energy intake did not differ among the groups. However, maternal CLA supplementation increased basal plasma adiponectin on d 3 and 4 of life during postprandial blood sampling. Adiponectin is known for its stimulatory effect on insulin sensitivity, which may also occur in calves (Havel, 2002; Liermann et al., 2020). This finding corresponds with the elevated RQUICKI in CLA calves at birth, as discussed above, and may indicate improved insulin sensitivity as a result of CLA supplementation. In contrast, CLA treatment, especially *trans*-10, *cis*-12 CLA, caused an

insulin-resistant state in rodents, although only at very high dosages (Halade et al., 2010; Bezan et al., 2018). Therefore, the insulin-sensitizing potential of CLA is still controversial (Benjamin et al., 2015).

Postnatal growth and development in calves is regulated by the somatotropic axis, which is in turn affected by protein and energy intake (Breier et al., 2000; Hammon et al., 2012). Because protein and energy intake was comparable among the study groups, treatment effects on plasma concentrations of growth hormone, IGF-I, and IGFBP in calves were rarely observed. However, the ratio of IGFBP-3 to IGFBP-2 on d 2 of life increased most in EFA calves. The ratio of IGFBP-3 to IGFBP-2 indicates the nutritional energy supply and is a good proxy for validating nutrient status in cattle (Breier et al., 2000; Renaville et al., 2002; Vogel et al., 2021). Whether elevated EFA status led to improved nutrient absorption in newborn calves could not be determined from the present study. However, our findings indicated increased glucose absorption shortly after feeding, and elevated intestinal glucose absorption was described in piglets when sows were supplemented with n-3 fatty acids (Gabler et al., 2007). These findings indicate improved intestinal function in neonates with elevated n-3 fatty acid status. Previous findings have indicated elevated plasma concentrations of IGF-I associated with higher plasma glucose levels in calves fed milk replacer enriched with EFA during the preweaning period (Garcia et al., 2014). Furthermore, plasma growth hormone was elevated after feeding on d 4 in the EFA calves in the present study, potentially indicating a stimulated somatotropic axis as a result of EFA treatment. In contrast, CLA calves had lower plasma IGF-I concentrations than non-CLA calves throughout the study. This finding was surprising, because CLA treatment increased plasma IGF-I in early lactation in the dams (Vogel et al., 2021). A stimulatory effect of CLA supplementation in dairy cows is also supported by further studies (Csillik et al., 2017), but CLA treatment reduced plasma IGF-I concentrations in growing rats (Li et al., 1999). Thus, variable effects of EFA and CLA on the somatotropic axis cannot be excluded, but the mechanisms behind these effects still need to be clarified.

## Conclusions

The present data indicate that maternal EFA and CLA supply influences the energy metabolism of neonatal calves. Elevated plasma concentrations of glucose and IGF-I immediately after birth before first colostrum intake indicate enhanced placental nutrient transfer and improved energetic status in calves when dams received EFA supplementation. In addition, maternal supplementation with EFA and CLA during late gestation affected the concentrations of endocrine factors such as leptin and adiponectin in colostrum, but these differences did not result in different plasma leptin and adiponectin concentrations in calves after first colostrum intake. However, maternal EFA and CLA supplementation influenced postnatal and postprandial changes associated with energy metabolism. Maternal EFA and CLA supplementation probably improved neonatal insulin response by enhancing plasma adiponectin and leptin, but it did not promote the somatotropic axis in a consistent manner. More research is necessary to clarify the effect of maternal EFA and CLA supplementation on the endocrine regulation of neonatal energy metabolism. Furthermore, maternal fatty acid supplementation had only minor effects on the growth of intestinal mucosa in calves.

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# CHAPTER 4

# General discussion

# 4. General discussion

The fatty acid supply of dairy cows fed modern corn-silage based rations has changed compared to pasture-based feeding, leading to a lower α-linolenic acid (ALA) and conjugated linoleic acid (CLA) provision and a higher intake of linoleic acid (LA) (Kay et al. 2005; Ferlay et al., 2006). Previous studies indicate that essential fatty acids (EFA) and CLA can modulate milk composition as well as aspects of the metabolism and development of mammals (Bauman et al., 1999; Ohnuki et al., 2001; Hill et al., 2009; Moallem, 2018). Whether a changed maternal EFA and CLA supply during gestation and lactation can affect the fatty acid status of the calf and aspects of its metabolism and development is still unclear, though. To address these knowledge gaps, the offspring from cows, which received a corn silage-based diet and abomasal supplementations of either coconut oil (CTRL), EFA, CLA or a combination of EFA and CLA (EFA+CLA) during late gestation and early lactation, were investigated in the present thesis. In the first study of this thesis (Chapter 2), the hypothesis was tested that these maternal treatments affect the plasma fatty acid composition of neonatal calves. Furthermore, it was tested whether maternal EFA and CLA supplementations change the nutrient composition of colostrum and transition milk and whether these maternal treatments can alter the body weight of the calves. The second study (Chapter 3) aimed to test the hypothesis that a changed maternal fatty acid supply affects aspects of the calves´ energy metabolism and endocrine growth regulation and that these effects are accompanied by alterations of the intestinal mucosa growth.

Results from the first study in this thesis (Chapter 2) suggest that a changed maternal EFA supply during late gestation can modulate the EFA status of the fetal calf as indicated by a slightly higher proportion of ALA in the plasma fatty acids of newborn, unsuckled calves from dams that received the ALA-rich EFA supplements. The only slightly higher plasma ALA levels as well as missing effects of maternal EFA supplementation on ALA concentrations in individual plasma lipid classes hint towards a limited placental ALA transfer, though, which is

in accordance with low LA transfers observed in previous studies (Noble et al., 1972; Noble et al., 1975; Garcia et al., 2014a). Nevertheless, maternal EFA supplementation seemed to promote the provision of ALA derivatives such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) for the fetus, presumably via desaturation of additive ALA in the placenta (Shand and Noble, 1981). These ALA derivatives are relevant for the development of the nervous system (DHA) and eicosanoid synthesis (EPA) and their levels were enhanced particularly in phospholipids (Koletzko et al., 2007), which serve as precursors for eicosanoids and can be taken up by the brain as lysophospholipids (Lagarde et al., 2001; Innis, 2005). Whether these changes of the fatty acid status coincide with modulations of the immune system or cognitive development might be subject of future studies. Higher *cis*-9, *trans*-11 CLA levels in plasma of EFA+CLA than CTRL calves suggest that CLA can be transferred to the fetal calf during gestation. However, low correlations between maternal and calf *cis*-9, *trans*-11 CLA and lacking correlations for the *trans*-10, *cis*-12 isomer indicate that the transfer of these fatty acids might be even more limited than placental EFA transfer and that it might vary between different CLA isomers. These results fortify the need for further research addressing the selective placental fatty acid transfer in cattle, which is widely unclear.

While the placental transfer seemed to enhance primarily the availability of ALA derivatives for calves from EFA supplemented dams, the transfer of ALA and CLA seemed to be accomplished particularly via the intake of colostrum and transition milk. Plasma levels of ALA and the two CLA isomers not only increased within the first five days of live irrespective of the group but levels of ALA and both CLA isomers could also be significantly enhanced in calves, whose dams received the respective fatty acids compared to calves whose dams did not receive them. A potential relevance of the LA supply via colostrum and milk to compensate for the low placental EFA transfer has been indicated in previous studies (Noble et al., 1972; Noble et al., 1975; Garcia et al., 2014a). Likewise, the intake of colostrum and milk from dams that were supplemented with ALA could aid to replenish the low ALA status directly after birth. This

might be of interest especially against the backdrop of the reduced ALA intake of cows that are fed common corn silage-based rations instead of pasture. Maternal ALA supplementation in the EFA group increased ALA proportions in colostrum fatty acids to a level that is slightly higher (1.34 vs 0.95 %) than proportions found in milk from grazing cows (Kay et al., 2005; Vogel et al., 2020). Whether feeding colostrum and milk from EFA supplemented dams is suitable to cover the EFA demand of neonatal calves cannot be concluded from the present thesis, though, as the EFA requirement of cattle is still unknown and demands further research.

However, results from the second study (Chapter 3) in this thesis suggest that a changed maternal EFA and CLA supply might not be without consequences for the energy metabolism of neonatal calves. Immediately after birth, the glucose status seemed to be improved by an enhanced maternal EFA supply during gestation as indicated by higher plasma glucose levels in EFA and EFA+CLA calves compared to CTRL and CLA. In comparison to plasma glucose levels of neonatal calves in previous studies, plasma glucose levels seemed to be enhanced in calves, whose dams received EFA (4 – 5.6 vs. 6.2 mmol/L) (Hammon and Blum, 1998; Steinhoff-Wagner et al., 2011; Gruse et al., 2016). These results might point towards an improved placental glucose transport in EFA supplemented dams but the underlying mechanisms are unclear and need to be elucidated in further studies. Especially in the early postnatal phase, an enhanced glucose supply might be relevant for calves, which often develop hypoglycemia (Hammon et al., 2012). Moreover, increased plasma glucose levels might have facilitated the simultaneously higher plasma insulin-like growth factor (IGF)-I levels in EFA compared to EFA+CLA calves (Gluckman and Pinal, 2003), which also suggests inhibiting effects of CLA on plasma IGF-I. Furthermore, inhibiting effects of CLA on IGF-I might have been amplified by an enhanced provision of n-3 fatty acids for EFA+CLA calves. Diverging CLA effects in dependence of additionally supplemented fatty acids were previously observed in rats that had lower serum levels of IGF-binding proteins (IGFBP), which comprised mainly IGFBP-3, when they received CLA in combination with n-3 fatty acids, whereas the

combination with n-6 fatty acids resulted in an increase of IGFBP levels (Li et al., 1999). Nevertheless, a significant interaction between EFA and CLA could not be detected for plasma IGF-I or IGFBP and interactions between EFA and CLA rarely occurred in the calves investigated in the present studies. Although the effects on glucose and IGF-I could indicate that maternal EFA supplementation partly promoted the energy status immediately after birth, these modulations were not sufficient to increase birth weights of EFA calves, though (Chapter 2).

While effects on the metabolism were repeatedly shown when EFA were fed directly to calves in previous studies (Hill et al., 2009; Garcia et al., 2014b), results of the second study (Chapter 3) in the present thesis suggest that aspects of the energy metabolism could also be modulated via the intake of colostrum and milk from EFA supplemented dams. In calves, consuming transition milk from EFA supplemented dams, ingested glucose seemed to be available faster as indicated by an earlier increase of plasma the [$^{13}C_6$]-glucose after this tracer was fed, potentially due to faster gastric emptying or an altered intestinal glucose absorption. Nevertheless, morphometric measurements did not suggest an altered intestinal absorption capacity as the intestinal mucosa was not affected by maternal EFA supplementation. Although a higher villus height to crypt depth ratio in the ileum of calves born from CLA supplemented dams shows that the intestinal morphometry can be partly modulated by maternal fatty acid supplementation, this modulation was not accompanied by signs of a higher glucose absorption, which is lowest in the ileum compared to other compartments of the small intestine (Bauer et al., 2001).

Results from the second study of this thesis (Chapter 3) suggest an impact of maternal EFA and CLA supplementation on the endocrine response to nutrient intake with higher postprandial insulin peaks in EFA and EFA+CLA calves that might have been facilitated by the aforementioned faster availability of ingested glucose. These effects on insulin could be responsible for the simultaneous modulations of the plasma non-esterified fatty acids (NEFA)

levels, which were lowest in EFA and CLA calves after feeding, pointing at a temporarily reduced mobilization of body fat (Hadorn et al., 1997). Furthermore, insulin sensitivity might have been stimulated in EFA calves that had higher plasma leptin levels on d 4 and 5, which can exert insulin sensitizing effects (Palou et al., 2018; Liermann et al., 2020). Concordantly, higher plasma adiponectin levels on d 3 and during postprandial sampling on d 4 might have facilitated insulin sensitivity in CLA calves (Havel, 2002). In addition to temporarily lower postprandial NEFA levels in plasma of CLA than control calves and despite similar insulin levels, a higher Revised Quantitative Insulin Sensitivity Check Index in calves from CLA supplemented dams directly after birth might also point towards insulin sensitizing effects of maternal CLA supplementation. However, the insulin sensitizing potential of CLA is still controversial and especially the impact of CLA on the energy metabolism of calves needs to be further elucidated.

Maternal EFA supplementation modulated single components of the somatotropic axis, which regulates growth and development and reacts to the energy and protein supply (Breier et al., 2000; Hammon et al., 2012). The supplemented fatty acids affected the composition of colostrum and transition milk only slightly and the intake of protein and energy was not enhanced in EFA calves. Nevertheless, in EFA calves, a temporary increase of the IGFBP-3/IGFBP-2 ratio was observed, which was previously associated with an enhanced nutritional status (Renaville et al., 2002; Vogel et al., 2021). However, the somatotropic axis did not seem to be consistently promoted by maternal EFA supplementation. Accordingly, maternal fatty acid supplementation did not affect the body weight or organ weight at the end of the trial. Thus, an improved performance, due to an enhanced maternal EFA supply could not be confirmed in the present thesis (Chapter 2) as opposed to previous studies reporting improved weight gains of calves after dietary EFA supplementation (Jenkins and Kramer, 1986; Hill et al., 2009; Garcia et al., 2014b). Moreover, an increased EFA supply in young age could exert its effects during the productive life as previously indicated by increased milk protein and fat

and numerically higher milk yields during first lactation (Garcia et al., 2016). Therefore, longer observation periods might be necessary to elucidate whether the aforementioned effects of maternal EFA and CLA supplementation are not only limited to aspects of the metabolism shortly after birth but might also affect the performance of calves in the longer term.

In conclusion, results of the present thesis demonstrate that a variable maternal EFA and CLA supply during gestation can reach the fetal calf and that particularly the intake of colostrum and transition milk from dams supplemented with these fatty acids can promote the EFA and CLA status of neonatal calves. Moreover, it was shown for the first time that not only the direct dietary supplementation of EFA but also an increased maternal supply with EFA or CLA during gestation and via colostrum and milk intake can modulate aspects of the calves' metabolism. This included signs of an improved energy status at birth after maternal EFA supplementation and indications of an intensified postprandial response in calves from EFA or CLA supplemented dams compared to the control group. Especially in the light of a reduced maternal ALA and CLA supply due to the common replacement of pasture by corn silage-based diets, these observations underline the need for further research addressing the impact of a changed maternal supply with these fatty acids over longer observation periods to exclude adverse effects on the energy metabolism and performance of the offspring with certainty.

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## Summary

## Summary

Present dairy nutrition often includes corn silage-based diets leading to a lower α-linolenic acid (ALA) and conjugated linoleic acid (CLA) availability and a higher linoleic acid (LA) provision compared to pasture. Effects of a changed dietary supply with the two essential fatty acids (EFA) ALA and LA and a changed CLA supply on aspects of the metabolism and development have been repeatedly shown in previous studies. However, whether an altered maternal supply with EFA and CLA can reach the calf during gestation or via the intake of milk and modulate its metabolism and development is still unclear. Thus, the present thesis aimed to investigate the impact of maternal supplementation with EFA, CLA, or both on the fatty acid status of neonatal calves and aspects of their energy metabolism and development.

In the first study of this thesis, 38 calves were investigated, which were born from dams receiving a corn silage-based diet and abomasal supplementations of either coconut oil (CTRL), EFA, CLA, or a combination of both (EFA+CLA) during late gestation and the subsequent lactation. During the trial comprising the first 5 d of life, calves received colostrum and transition milk from their own dam. To determine the fatty acid status, fatty acids were analyzed in plasma sampled immediately after birth and on d 5 of life. It was shown that maternal supplementation with the ALA-rich EFA supplement and CLA can enhance proportions of ALA and *cis*-9, *trans*-11 CLA in plasma lipids of newborn, unsuckled calves. Particularly the intake of colostrum and transition milk contributed to the fatty acid transfer, which resulted in an increase of ALA as well as *cis*-9, *trans*-11 and *trans*-10, *cis*-12 CLA proportions during the first days of life and the increase of ALA and CLA was more pronounced in calves, whose dams received these fatty acids. However, body weights of the calves were not affected by maternal supplementation.

The second study of this thesis addressed the calves´ energy metabolism and development. Therefore, metabolites and hormones with relevance for energy metabolism and endocrine

growth regulation were analyzed in plasma samples taken daily before feeding and after feeding on day 4 of life. After slaughter on day 5 of life, the intestinal mucosa was investigated by histomorphometry. Elevated plasma glucose and insulin-like growth factor-I levels immediately after birth in EFA calves suggest a modified energy status due to maternal EFA supplementation during gestation. Maternal EFA and CLA supplementation seemed to affect the insulin response, leading to temporarily lower non-esterified fatty acid levels in plasma of EFA and CLA calves after feeding, which might have been facilitated by increased plasma concentrations of leptin or adiponectin. However, the somatotropic axis was not consistently modulated and only minor effects on the intestinal mucosa were induced by the maternal supplementation.

In conclusion, results in the present thesis show that an altered maternal supply with EFA and CLA can reach the calf during gestation and via the intake of colostrum and transition milk. Moreover, an altered maternal EFA and CLA supply can modulate the energy metabolism of neonatal calves. While maternal EFA supplementation might promote the energy status immediately after birth, supplementing EFA or CLA to dams could enhance the postprandial response of their calves. However, maternal fatty acid supplementation induced only minor effects on the early postnatal development.

## Zusammenfassung

In der gegenwärtigen Milchviehfütterung werden häufig maissilagebasierte Rationen eingesetzt, die weniger α-Linolensäure (ALA) und konjugierte Linolsäure (CLA) und mehr Linolsäure (LA) liefern als die weidebasierte Fütterung. Einflüsse einer veränderten Aufnahme der beiden essentiellen Fettsäuren (EFA) ALA und LA sowie einer veränderten CLA Aufnahme auf Aspekte des Stoffwechsels und der Entwicklung wurden wiederholt in vorherigen Studien gezeigt. Ob eine veränderte maternale Versorgung mit EFA und CLA das Kalb während der Trächtigkeit oder über die Milchaufnahme erreichen und seinen Stoffwechsel oder seine Entwicklung beeinflussen kann, ist jedoch weiterhin unklar. Somit war es das Ziel der vorliegenden Arbeit, den Einfluss der maternalen Supplementation mit EFA, CLA oder beidem zusammen auf den Fettsäurestatus von neugeborenen Kälbern und Aspekte ihres Energiestoffwechsels und ihrer Entwicklung zu untersuchen.

In der ersten Studie dieser Arbeit wurden 38 Kälber untersucht, welche von Müttern abstammten, die maissilagebasierte Rationen bekamen und entweder mit Kokosöl (CTRL), EFA, CLA oder einer Kombination von beidem während der Spätträchtigkeit und der folgenden Laktation supplementiert wurden. Während des Versuchs, der die ersten fünf Lebestage umfasste, erhielten die Kälber Kolostrum und Transitmilch ihrer eigenen Mutter. Um den Fettsäurestatus zu bestimmen, wurden die Fettsäuren in Plasmaproben bestimmt, die unmittelbar nach der Geburt und am fünften Lebenstag genommen wurden. Es zeigte sich, dass die maternale Supplementation mit dem ALA-reichen EFA-Supplement und CLA die Anteile von ALA und *cis*-9, *trans*-11 CLA in den Plasmalipiden von neugeborenen Kälbern vor der ersten Fütterung erhöhen kann. Besonders die Aufnahme von Kolostrum und Transitmilch trug jedoch zum Fettsäuretransfer bei, was zu einer Erhöhung der Anteile von ALA sowie *cis*-9, *trans*-11 CLA und *trans*-10, *cis*-12 CLA während der ersten Lebenstage führte, die

ausgeprägter in Kälbern war, deren Mütter diese Fettsäuren bekamen. Die Körpergewichte der Kälber wurden hingegen nicht von der maternalen Supplementation beeinflusst.

Die zweite Studie dieser Arbeit befasste sich mit dem Energiestoffwechsel und der Entwicklung der Kälber. Hierzu wurden Stoffwechselprodukte und Hormone mit Relevanz für den Energiestoffwechsel und die endokrine Regulation des Wachstums in Plasmaproben untersucht, die täglich vor der Fütterung und nach der Fütterung an Tag vier genommen wurden. Nach der Schlachtung an Tag fünf wurde die intestinale Mukosa entnommen und histomorphometrisch untersucht. Erhöhte Plasmaspiegel von Glukose und insulinähnlichem Wachstumsfaktor-I in EFA-Kälbern direkt nach der Geburt deuten auf einen veränderten Energiestatus durch die maternale EFA Supplementation während der Trächtigkeit hin. Die maternale Supplementation mit EFA und CLA schien die Insulinantwort zu beeinflussen. So war der Spiegel der unveresterten Fettsäuren im Plasma von EFA- und CLA-Kälbern nach der Fütterung zeitweise verringert, was möglicherweise durch eine verbesserte Insulinwirkung aufgrund erhöhter Plasmakonzentrationen von Leptin oder Adiponektin hervorgerufen wurde. Die somatotrope Achse wurde jedoch nicht eindeutig verändert und der Einfluss der maternalen Supplementation auf die intestinale Mukosa war gering.

Zusammenfassend lässt sich aus den Ergebnissen dieser Arbeit ableiten, dass eine veränderte mütterliche Versorgung mit EFA und CLA das Kalb während der Trächtigkeit und über die Aufnahme von Kolostrum und Transitmilch erreichen kann. Zusätzlich kann eine veränderte mütterliche Versorgung mit EFA und CLA den Energiestoffwechsel von neugeborenen Kälbern beeinflussen. Während die maternale EFA Supplementation den Energiestatus unmittelbar nach der Geburt positiv zu beeinflussen scheint, kann die postprandiale Insulinwirkung bei den Kälbern offenbar verstärkt werden, deren Mütter EFA oder CLA erhalten. Die maternale Fettsäuresupplementation hat jedoch nur geringe Effekte auf die frühpostnatale Entwicklung.

# Appendix

APPENDIX

**Supplemental Table S2.1: Effects of the maternal supplementation with coconut oil (CTRL; n = 9), linseed and safflower oil (EFA; n = 9), Lutalin (CLA; *cis*-9, *trans*-11 and *trans*-10, *cis*-12; n = 9), or a combination of the EFA and CLA supplement (EFA+CLA; n = 11) on the fatty acid composition in plasma fat of calves on d 1 and 5 of life[1]**

| | | Maternal supplementation | | | | *P*-value[2] | | |
|---|---|---|---|---|---|---|---|---|
| Fatty acid, %[3] | Time[4] | CTRL | EFA | CLA | EFA+CLA | EFA<br>Time | CLA<br>EFA × time | EFA × CLA<br>CLA × time |
| Plasma lipid content | 1 | 0.08 ± 0.01[B] | 0.06 ± 0.01[B] | 0.06 ± 0.01[B] | 0.06 ± 0.01[B] | 0.46 | 0.37 | 0.68 |
| | 5 | 0.18 ± 0.01[A] | 0.17 ± 0.01[A] | 0.17 ± 0.01[A] | 0.17 ± 0.01[A] | <0.001 | 0.66 | 0.80 |
| 10:0 | 1 | 0.22 ± 0.11 | 0.22 ± 0.11 | 0.30 ± 0.11 | 0.36 ± 0.10 | 0.98 | 0.38 | 0.93 |
| | 5 | 0.22 ± 0.05 | 0.24 ± 0.05 | 0.25 ± 0.05 | 0.19 ± 0.04 | 0.35 | 0.68 | 0.28 |
| 11:0 | 1 | 0.02 ± 0.01 | 0.01 ± 0.01 | 0.02 ± 0.01 | 0.02 ± 0.01 | 0.64 | 0.88 | 0.65 |
| | 5 | 0.01 ± 0.01 | 0.02 ± 0.00 | 0.01 ± 0.00 | 0.01 ± 0.00 | 0.23 | 0.56 | 0.25 |
| 12:0 | 1 | 0.66 ± 0.13 | 0.41 ± 0.12 | 0.50 ± 0.13 | 0.40 ± 0.11 | 0.02 | 0.13 | 0.76 |
| | 5 | 0.84 ± 0.12 | 0.70 ± 0.12 | 0.75 ± 0.12 | 0.53 ± 0.10 | <0.01 | 0.95 | 0.73 |
| 13:0 | 1 | 0.02 ± 0.01 | 0.01 ± 0.01 | 0.03 ± 0.01 | 0.01 ± 0.01 | <0.01 | 0.90 | 0.21 |
| | 5 | 0.03 ± 0.00 | 0.03 ± 0.00 | 0.03 ± 0.00 | 0.02 ± 0.00 | 0.04 | 0.45 | 0.15 |
| 14:0 | 1 | 2.17 ± 0.49[B] | 1.26 ± 0.46[B] | 1.78 ± 0.48[B] | 1.40 ± 0.40[B] | 0.03 | 0.79 | 0.81 |
| | 5 | 4.07 ± 0.52[A] | 3.84 ± 0.49[A] | 4.44 ± 0.51[A] | 3.43 ± 0.43[A] | <0.001 | 0.96 | 0.84 |
| 14:1 *cis*-9 | 1 | 0.37 ± 0.07 | 0.23 ± 0.06 | 0.32 ± 0.07 | 0.20 ± 0.06 | 0.01 | 0.17 | 0.69 |
| | 5 | 0.26 ± 0.04[a] | 0.18 ± 0.04[ab] | 0.19 ± 0.04[ab] | 0.14 ± 0.03[b] | <0.01 | 0.30 | 0.71 |
| 15:0 | 1 | 0.73 ± 0.08 | 0.67 ± 0.08 | 0.67 ± 0.08 | 0.63 ± 0.07 | 0.12 | 0.56 | 0.74 |
| | 5 | 0.55 ± 0.04 | 0.51 ± 0.04 | 0.59 ± 0.04 | 0.48 ± 0.03 | <0.01 | 0.86 | 0.48 |
| 16:0 | 1 | 26.8 ± 1.3[A] | 24.4 ± 1.2 | 25.8 ± 1.2 | 24.9 ± 1.0[A] | 0.03 | 0.58 | 0.97 |
| | 5 | 22.5 ± 1.4[B] | 21.3 ± 1.3 | 22.6 ± 1.3 | 20.0 ± 1.1[B] | <0.001 | 0.86 | 0.72 |

**Supplemental Table S2.1: Continuation**

| Fatty acid, %[3] | Time[4] | Maternal supplementation | | | | *P*-value[2] | | |
|---|---|---|---|---|---|---|---|---|
| | | CTRL | EFA | CLA | EFA+CLA | EFA<br>Time | CLA<br>EFA × time | EFA × CLA<br>CLA × time |
| 16:1*cis*-9 | 1 | 5.02 ± 0.38$^{A}$ | 4.98 ± 0.37$^{A}$ | 4.85 ± 0.38$^{A}$ | 4.54 ± 0.33$^{A}$ | 0.01 | 0.14 | 0.52 |
| | 5 | 3.40 ± 0.26$^{a,B}$ | 2.61 ± 0.24$^{bc,B}$ | 3.23 ± 0.25$^{ab,B}$ | 2.21 ± 0.21$^{c,B}$ | <0.001 | 0.03 | 0.94 |
| 17:0 | 1 | 1.17 ± 0.06$^{A}$ | 1.20 ± 0.05$^{A}$ | 1.19 ± 0.05$^{A}$ | 1.22 ± 0.05$^{A}$ | 0.32 | 0.60 | 0.81 |
| | 5 | 0.86 ± 0.04$^{B}$ | 0.76 ± 0.04$^{B}$ | 0.85 ± 0.04$^{B}$ | 0.78 ± 0.03$^{B}$ | <0.001 | 0.02 | 0.68 |
| 17:1*cis*-9 | 1 | 0.84 ± 0.05$^{A}$ | 0.88 ± 0.04$^{A}$ | 0.88 ± 0.05$^{A}$ | 0.87 ± 0.04$^{A}$ | <0.01 | 0.48 | 0.67 |
| | 5 | 0.66 ± 0.04$^{a,B}$ | 0.48 ± 0.03$^{b,B}$ | 0.60 ± 0.03$^{a,B}$ | 0.44 ± 0.03$^{b,B}$ | <0.001 | <0.001 | 0.12 |
| 18:0 | 1 | 11.5 ± 0.6 | 11.7 ± 0.5 | 11.8 ± 0.6 | 11.4 ± 0.5 | 0.83 | 0.03 | 0.19 |
| | 5 | 11.0 ± 0.3$^{c}$ | 11.6 ± 0.3$^{bc}$ | 12.5 ± 0.3$^{a}$ | 12.3 ± 0.3$^{ab}$ | 0.34 | 0.63 | 0.06 |
| 18:1*cis*-9 | 1 | 24.1 ± 1.2 | 25.1 ± 1.1$^{A}$ | 24.0 ± 1.1$^{A}$ | 23.8 ± 1.0$^{A}$ | <0.01 | 0.03 | 0.95 |
| | 5 | 21.2 ± 1.0$^{a}$ | 16.1 ± 1.0$^{bc,B}$ | 18.8 ± 1.0$^{ab,B}$ | 14.9 ± 0.9$^{c,B}$ | <0.001 | <0.001 | 0.43 |
| 18:1*cis*-11 | 1 | 3.79 ± 0.18$^{A}$ | 4.21 ± 0.17$^{A}$ | 4.32 ± 0.18$^{A}$ | 3.92 ± 0.15$^{A}$ | 0.34 | 0.60 | 0.03 |
| | 5 | 1.89 ± 0.15$^{a,B}$ | 1.69 ± 0.13$^{ab,B}$ | 1.67 ± 0.14$^{ab,B}$ | 1.47 ± 0.12$^{b,B}$ | <0.001 | 0.18 | 0.03 |
| 18:1*trans*-9 | 1 | 0.09 ± 0.02$^{ab,B}$ | 0.05 ± 0.02$^{b,B}$ | 0.12 ± 0.02$^{a,B}$ | 0.08 ± 0.02$^{ab,B}$ | <0.001 | 0.02 | 0.09 |
| | 5 | 0.28 ± 0.03$^{b,A}$ | 0.24 ± 0.02$^{b,A}$ | 0.37 ± 0.03$^{a,A}$ | 0.23 ± 0.02$^{b,A}$ | <0.001 | 0.01 | 0.61 |
| 18:1*trans*-11 | 1 | 0.04 ± 0.02$^{B}$ | 0.03 ± 0.02$^{B}$ | 0.02 ± 0.02$^{B}$ | 0.02 ± 0.01$^{B}$ | 0.39 | 0.54 | 0.79 |
| | 5 | 0.50 ± 0.04$^{A}$ | 0.47 ± 0.04$^{A}$ | 0.49 ± 0.04$^{A}$ | 0.45 ± 0.04$^{A}$ | <0.001 | 0.47 | 0.94 |
| 18:2 *cis*-9, *cis*-12 | 1 | 4.96 ± 0.89$^{B}$ | 5.95 ± 0.81$^{B}$ | 5.41 ± 0.86$^{B}$ | 6.69 ± 0.70$^{B}$ | 0.11 | 0.22 | 0.50 |
| (LA) | 5 | 21.28 ± 1.62$^{A}$ | 22.11 ± 1.57$^{A}$ | 21.88 ± 1.60$^{A}$ | 24.84 ± 1.41$^{A}$ | <0.001 | 0.54 | 0.38 |
| 18:2 *cis*-9, *trans*-11 | 1 | 0.07 ± 0.01$^{bc,B}$ | 0.06 ± 0.01$^{c,B}$ | 0.10 ± 0.01$^{ab,B}$ | 0.10 ± 0.01$^{a,B}$ | 0.03 | <0.001 | 0.71 |
| CLA | 5 | 0.21 ± 0.02$^{b,A}$ | 0.16 ± 0.02$^{b,A}$ | 0.35 ± 0.02$^{a,A}$ | 0.31 ± 0.02$^{a,A}$ | <0.001 | <0.01 | <0.001 |
| 18:2 *trans*-10, *cis*-12 | 1 | 0.03 ± 0.01 | 0.03 ± 0.01 | 0.02 ± 0.01$^{B}$ | 0.02 ± 0.01$^{B}$ | 0.60 | <0.001 | 0.82 |
| CLA | 5 | 0.00 ± 0.01$^{b*}$ | 0.01 ± 0.01$^{b}$ | 0.15 ± 0.01$^{a,A}$ | 0.16 ± 0.01$^{a,A}$ | <0.001 | 0.72 | <0.001 |
| 18:3 *cis*-6, *cis*-9, *cis*-12 | 1 | 0.34 ± 0.05 | 0.35 ± 0.05$^{A}$ | 0.34 ± 0.05 | 0.40 ± 0.04$^{A}$ | 0.10 | 0.99 | 0.87 |
| | 5 | 0.35 ± 0.04$^{a}$ | 0.24 ± 0.04$^{ab,B}$ | 0.35 ± 0.04$^{a}$ | 0.19 ± 0.03$^{b,B}$ | <0.001 | <0.001 | 0.19 |

**Supplemental Table S2.1: Continuation**

| Fatty acid, %[3] | Time[4] | Maternal supplementation | | | | *P*-value[2] | | |
|---|---|---|---|---|---|---|---|---|
| | | CTRL | EFA | CLA | EFA+CLA | EFA<br>Time | CLA<br>EFA × time | EFA × CLA<br>CLA × time |
| 18:3 *cis*-9, *cis*-12, *cis*-15 | 1 | 0.14 ± 0.12$^{b,B}$ | 0.55 ± 0.11$^{a,B}$ | 0.16 ± 0.12$^{b,B}$ | 0.66 ± 0.09$^{a,B}$ | <0.001 | 0.23 | 0.34 |
| (ALA) | 5 | 1.43 ± 0.32$^{b,A}$ | 7.62 ± 0.32$^{a,A}$ | 1.49 ± 0.32$^{b,A}$ | 8.21 ± 0.29$^{a,A}$ | <0.001 | <0.001 | 0.39 |
| 18:4 *cis*-6, *cis*-9, | 1 | 0.01 ± 0.02$^{B}$ | 0.00 ± 0.02$^{B*}$ | 0.03 ± 0.02 | 0.02 ± 0.01 | 0.29 | 0.7 | 0.78 |
| *cis*-12, *cis*-15 | 5 | 0.05 ± 0.01$^{A}$ | 0.06 ± 0.01$^{A}$ | 0.05 ± 0.01 | 0.03 ± 0.01 | <0.001 | 0.85 | 0.02 |
| 20:0 | 1 | 0.49 ± 0.06$^{A}$ | 0.41 ± 0.06$^{A}$ | 0.48 ± 0.06$^{A}$ | 0.39 ± 0.05$^{A}$ | 0.17 | 0.99 | 0.87 |
| | 5 | 0.13 ± 0.01$^{B}$ | 0.13 ± 0.01$^{B}$ | 0.15 ± 0.01$^{B}$ | 0.14 ± 0.01$^{B}$ | <0.001 | 0.18 | 0.58 |
| 20:1*cis*-11 | 1 | 0.13 ± 0.02 | 0.14 ± 0.02 | 0.13 ± 0.02 | 0.13 ± 0.02 | 0.76 | 0.50 | 0.48 |
| | 5 | 0.16 ± 0.02 | 0.13 ± 0.02 | 0.14 ± 0.02 | 0.14 ± 0.01 | 0.53 | 0.20 | 0.99 |
| 20:2, *cis*-11, *cis*-14 | 1 | 0.13 ± 0.02 | 0.13 ± 0.02 | 0.15 ± 0.02 | 0.17 ± 0.02 | 0.73 | <0.001 | 0.91 |
| | 5 | 0.11 ± 0.01$^{bc}$ | 0.10 ± 0.01$^{c}$ | 0.16 ± 0.01$^{a}$ | 0.14 ± 0.01$^{ab}$ | <0.01 | 0.10 | 0.36 |
| 20:3, *cis*-8, *cis*-11, | 1 | 2.79 ± 0.29$^{A}$ | 3.04 ± 0.28$^{A}$ | 2.55 ± 0.28$^{A}$ | 3.25 ± 0.25$^{A}$ | 0.42 | 0.61 | 0.45 |
| *cis*-14 | 5 | 1.32 ± 0.11$^{a,B}$ | 1.09 ± 0.10$^{ab,B}$ | 1.18 ± 0.10$^{ab,B}$ | 0.96 ± 0.08$^{b,B}$ | <0.001 | <0.01 | 0.63 |
| 20:3, *cis*-11, *cis*-14, | 1 | 0.00 ± 0.01$^{b*}$ | 0.03 ± 0.01$^{ab,B}$ | 0.01 ± 0.01$^{b}$ | 0.05 ± 0.01$^{a,B}$ | <0.001 | <0.01 | 0.02 |
| *cis*-17 | 5 | 0.01 ± 0.01$^{c}$ | 0.09 ± 0.01$^{b,A}$ | 0.01 ± 0.01$^{c}$ | 0.13 ± 0.01$^{a,A}$ | <0.001 | <0.001 | 0.62 |
| 20:4 *cis*-5, *cis*-8, *cis*-11, | 1 | 6.85 ± 0.57$^{A}$ | 5.57 ± 0.55$^{A}$ | 7.16 ± 0.56$^{A}$ | 6.12 ± 0.49$^{A}$ | <0.01 | 0.76 | 0.64 |
| *cis*-14 (ARA) | 5 | 3.96 ± 0.32$^{a,B}$ | 2.98 ± 0.29$^{b,B}$ | 3.55 ± 0.31$^{ab,B}$ | 2.90 ± 0.25$^{b,B}$ | <0.001 | 0.46 | 0.15 |
| 20:5 *cis*-5, *cis*-8, *cis*-11, | 1 | 0.33 ± 0.13$^{b}$ | 1.27 ± 0.12$^{a}$ | 0.34 ± 0.13$^{b}$ | 1.53 ± 0.11$^{a}$ | <0.001 | 0.55 | 0.44 |
| *cis*-14, *cis*-17 (EPA) | 5 | 0.54 ± 0.13$^{b}$ | 1.45 ± 0.12$^{a}$ | 0.51 ± 0.12$^{b}$ | 1.40 ± 0.10$^{a}$ | 0.02 | 0.10 | 0.07 |
| 21:0 | 1 | 0.22 ± 0.04$^{A}$ | 0.18 ± 0.04$^{A}$ | 0.21 ± 0.04$^{A}$ | 0.17 ± 0.03$^{A}$ | 0.40 | 0.89 | 0.90 |
| | 5 | 0.04 ± 0.01$^{B}$ | 0.05 ± 0.01$^{B}$ | 0.05 ± 0.01$^{B}$ | 0.05 ± 0.01$^{B}$ | <0.001 | 0.20 | 0.55 |
| 22:0 | 1 | 0.91 ± 0.13$^{A}$ | 0.76 ± 0.13$^{A}$ | 0.90 ± 0.13$^{A}$ | 0.73 ± 0.12$^{A}$ | 0.29 | 0.98 | 0.89 |
| | 5 | 0.17 ± 0.03$^{B}$ | 0.19 ± 0.03$^{B}$ | 0.20 ± 0.03$^{B}$ | 0.21 ± 0.03$^{B}$ | <0.001 | 0.18 | 0.74 |
| 22:1, *cis*-13 | 1 | 0.00 ± 0.00$^{*}$ | 0.00 ± 0.00$^{*}$ | 0.00 ± 0.00$^{B*}$ | 0.00 ± 0.00$^{*}$ | 0.07 | 0.78 | 0.56 |
| | 5 | 0.01 ± 0.00 | 0.01 ± 0.00 | 0.02 ± 0.00$^{A}$ | 0.01 ± 0.00 | <0.001 | 0.26 | 0.60 |

**Supplemental Table S2.1: Continuation**

| Fatty acid, %[3] | Time[4] | Maternal supplementation | | | | *P*-value[2] | | |
|---|---|---|---|---|---|---|---|---|
| | | CTRL | EFA | CLA | EFA+CLA | EFA<br>Time | CLA<br>EFA × time | EFA × CLA<br>CLA × time |
| 22:2, *cis*-13, *cis*-16 | 1 | 0.01 ± 0.06 | 0.10 ± 0.06 | 0.04 ± 0.06 | 0.07 ± 0.05 | 0.15 | 0.80 | 0.34 |
| | 5 | 0.00 ± 0.03* | 0.06 ± 0.03 | 0.02 ± 0.03 | 0.02 ± 0.03 | 0.20 | 0.54 | 0.89 |
| 22:4 *cis*-7, *cis*-10, *cis*- | 1 | 0.48 ± 0.04$^{a,A}$ | 0.25 ± 0.04$^{b,A}$ | 0.54 ± 0.04$^{a,A}$ | 0.26 ± 0.03$^{b,A}$ | <0.001 | 0.34 | 0.48 |
| 13, *cis*-16 | 5 | 0.23 ± 0.02$^{a,B}$ | 0.12 ± 0.01$^{b,B}$ | 0.24 ± 0.02$^{a,B}$ | 0.12 ± 0.01$^{b,B}$ | <0.001 | <0.001 | 0.30 |
| 22:5 *cis*-4, *cis*-7, *cis*-10, | 1 | 0.14 ± 0.02$^{ab}$ | 0.06 ± 0.02$^{bc}$ | 0.16 ± 0.02$^{a,A}$ | 0.04 ± 0.02$^{c}$ | <0.001 | 0.89 | 0.19 |
| *cis*-13, *cis*-16 | 5 | 0.07 ± 0.01$^{a}$ | 0.03 ± 0.01$^{b}$ | 0.08 ± 0.01$^{a,B}$ | 0.02 ± 0.01$^{b}$ | <0.001 | 0.04 | 0.94 |
| 22:5 *cis*-7, *cis*-10, *cis*-13, | 1 | 1.44 ± 0.16$^{A}$ | 1.88 ± 0.16$^{A}$ | 1.39 ± 0.16$^{A}$ | 1.85 ± 0.14$^{A}$ | <0.001 | 0.84 | 0.81 |
| *cis*-16, *cis*-19 (DPA) | 5 | 0.47 ± 0.07$^{b,B}$ | 0.84 ± 0.06$^{a,B}$ | 0.45 ± 0.07$^{b,B}$ | 0.88 ± 0.05$^{a,B}$ | <0.001 | 0.71 | 0.70 |
| 22:6 *cis*-4, *cis*-7, *cis*-10, | 1 | 1.04 ± 0.18$^{b,A}$ | 1.88 ± 0.18$^{a,A}$ | 1.03 ± 0.18$^{b,A}$ | 1.85 ± 0.16$^{a,A}$ | <0.001 | 0.81 | 0.88 |
| *cis*-13, *cis*-16, *cis*-19 (DHA) | 5 | 0.32 ± 0.09$^{b,B}$ | 0.61 ± 0.08$^{a,B}$ | 0.31 ± 0.08$^{b,B}$ | 0.57 ± 0.07$^{a,B}$ | <0.001 | <0.001 | 0.96 |
| 23:0 | 1 | 0.42 ± 0.07$^{a,A}$ | 0.20 ± 0.07$^{ab}$ | 0.26 ± 0.07$^{ab}$ | 0.18 ± 0.06$^{b}$ | 0.02 | 0.30 | 0.35 |
| | 5 | 0.04 ± 0.05$^{B}$ | 0.01 ± 0.04 | 0.05 ± 0.05 | 0.03 ± 0.04 | <0.001 | 0.06 | 0.13 |
| 24:0 | 1 | 0.97 ± 0.13$^{A}$ | 0.82 ± 0.13$^{A}$ | 0.93 ± 0.13$^{A}$ | 0.78 ± 0.12$^{A}$ | 0.29 | 0.91 | 0.98 |
| | 5 | 0.18 ± 0.04$^{B}$ | 0.19 ± 0.03$^{B}$ | 0.20 ± 0.03$^{B}$ | 0.22 ± 0.03$^{B}$ | <0.001 | 0.20 | 0.66 |
| SFA[5] | 1 | 46.2 ± 1.6$^{A}$ | 42.4 ± 1.4 | 45.2 ± 1.5 | 43.0 ± 1.2 | <0.01 | 0.81 | 0.67 |
| | 5 | 40.7 ± 1.7$^{B}$ | 39.8 ± 1.6 | 43.0 ± 1.7 | 38.9 ± 1.4 | <0.001 | 0.74 | 0.56 |
| MUFA[6] | 1 | 34.3 ± 1.4$^{A}$ | 35.6 ± 1.4$^{A}$ | 34.6 ± 1.4$^{A}$ | 33.4 ± 1.2$^{A}$ | <0.001 | 0.02 | 0.59 |
| | 5 | 28.4 ± 1.3$^{a,B}$ | 21.8 ± 1.2$^{bc,B}$ | 25.4 ± 1.2$^{ab,B}$ | 19.9 ± 1.0$^{c,B}$ | <0.001 | <0.001 | 0.37 |
| PUFA[7] | 1 | 19.8 ± 2.0$^{B}$ | 22.7 ± 1.8$^{B}$ | 21.1 ± 1.9$^{B}$ | 24.4 ± 1.6$^{B}$ | <0.001 | 0.16 | 0.63 |
| | 5 | 31.3 ± 2.4$^{c,A}$ | 39.1 ± 2.2$^{ab,A}$ | 32.4 ± 2.3$^{bc,A}$ | 42.2 ± 2.0$^{a,A}$ | <0.001 | <0.01 | 0.76 |
| Sum of n-3 fatty acids[8] | 1 | 3.22 ± 0.41$^{b}$ | 5.88 ± 0.39$^{a,B}$ | 3.28 ± 0.40$^{b}$ | 6.28 ± 0.34$^{a,B}$ | <0.001 | 0.20 | 0.30 |
| | 5 | 3.09 ± 0.44$^{b}$ | 10.94 ± 0.42$^{a,A}$ | 3.15 ± 0.43$^{b}$ | 11.55 ± 0.37$^{a,A}$ | <0.001 | <0.001 | 0.83 |
| Sum of n-6 fatty acids[9] | 1 | 16.4 ± 1.7$^{B}$ | 16.6 ± 1.5$^{B}$ | 17.6 ± 1.6$^{B}$ | 18.0 ± 1.3$^{B}$ | 0.68 | 0.22 | 0.73 |
| | 5 | 28.0 ± 2.0$^{A}$ | 27.9 ± 1.9$^{A}$ | 28.7 ± 2.0$^{A}$ | 30.1 ± 1.7$^{A}$ | <0.001 | 0.81 | 0.89 |

**Supplemental Table S2.1: Continuation**

| Fatty acid, %[3] | Time[4] | Maternal supplementation | | | | *P*-value[2] | | |
|---|---|---|---|---|---|---|---|---|
| | | CTRL | EFA | CLA | EFA+CLA | EFA<br>Time | CLA<br>EFA × time | EFA × CLA<br>CLA × time |
| Ratio n-6 : n-3 fatty acids | 1 | 5.18 ± 0.31[a,B] | 2.97 ± 0.28[b] | 5.52 ± 0.30[a,B] | 2.91 ± 0.24[b] | <0.001 | 0.55 | 0.36 |
| | 5 | 9.42 ± 0.41[a,A] | 2.70 ± 0.39[b] | 9.73 ± 0.40[a,A] | 2.62 ± 0.34[b] | <0.001 | <0.001 | 0.94 |
| Δ-5 desaturase index[10] | 1 | 0.71 ± 0.02[a] | 0.64 ± 0.02[b,B] | 0.73 ± 0.02[a] | 0.65 ± 0.01[b,B] | <0.001 | 0.15 | 0.89 |
| | 5 | 0.74 ± 0.01 | 0.73 ± 0.01[A] | 0.75 ± 0.01 | 0.75 ± 0.01[A] | <0.001 | <0.001 | 0.58 |
| Δ-6 desaturase index[11] | 1 | 0.39 ± 0.02[A] | 0.36 ± 0.02[A] | 0.36 ± 0.02[A] | 0.35 ± 0.02[A] | 0.22 | 0.23 | 0.79 |
| | 5 | 0.06 ± 0.01[a,B] | 0.05 ± 0.01[ab,B] | 0.05 ± 0.01[ab,B] | 0.04 ± 0.01[b,B] | <0.001 | 0.97 | 0.56 |
| Δ-9 desaturase index[12] | 1 | 0.68 ± 0.02 | 0.68 ± 0.02[A] | 0.67 ± 0.02 | 0.67 ± 0.01[A] | <0.001 | <0.001 | 0.32 |
| | 5 | 0.66 ± 0.01[a] | 0.58 ± 0.01[bc,B] | 0.60 ± 0.01[b] | 0.55 ± 0.01[c,B] | <0.001 | <0.01 | 0.12 |

[a-c] LSM within a row with different lowercase letters differ between treatments ($P < 0.05$).

[A, B] LSM within a column with different uppercase letters differ between days of life ($P < 0.05$).

[*] LSM were below the detection limit of 0.01%.

[1] Values are presented as LSM ± SE.

[2] *P*-values for fixed effects are presented in 2 rows: The first row indicates *P*-values for the effect of EFA, CLA, and their interaction; the second row indicates *P*-values for the effect of time and interactions between EFA or CLA and time.

[3] Proportion of total lipids in plasma; proportion of the presented fatty acid in total plasma fatty acids.

[4] Day of life.

[5] Sum of saturated fatty acids, consisting of 10:0; 11:0; 12:0; 13:0; 14:0; 15:0; 16:0; 17:0; 18:0; 20:0; 21:0; 22:0; 23:0; 24:0.

[6] Sum of monounsaturated fatty acids, consisting of 14:1 *cis*-9; 16:1 *cis*-9; 17:1 *cis*-9; 18:1 *cis*-9; 18:1 *cis*-11; 18:1 *trans*-9; 18:1 *trans*-11; 20:1*cis*-11; 22:1 *cis*-13.

[7] Sum of polyunsaturated fatty acids, consisting of 18:2 *cis*-9, *cis*-12; 18:2 *cis*-9, *trans*-11; 18:2 *trans*-10, *cis*-12; 18:2 *trans*-9, *trans*-12; 18:3 *cis*-6, *cis*-9, *cis*-12; 18:3 *cis*-9, *cis*-12, *cis*-15; 18:4 *cis*-6, *cis*-9, *cis*-12, *cis*-15; 20:2 *cis*-11, *cis*-14; 20:3 *cis*-8, *cis*-11, *cis*-14; 20:3 *cis*-11, *cis*-14, *cis*-17; 20:4 *cis*-5, *cis*-8, *cis*-11, *cis*-14; 20:5 *cis*-5, *cis*-8, *cis*-11, *cis*-14, *cis*-17; 22:2 *cis*-13, *cis*-16; 22:4 *cis*-7, *cis*-10, *cis*-13, *cis*-16; 22:5 *cis*-4, *cis*-7, *cis*-10, *cis*-13, *cis*-16; 22:5 *cis*-7, *cis*-10, *cis*-13, *cis*-16, *cis*-19; 22:6 *cis*-4, *cis*-7, *cis*-10, *cis*-13, *cis*-16, *cis*-19.

[8] Sum of n-3 fatty acids, consisting of 18:3 *cis*-9, *cis*-12, *cis*-15; 18:4 *cis*-6, *cis*-9, *cis*-12, *cis*-15; 20:3, *cis*-11, *cis*-14, *cis*-17; 20:5 *cis*-5, *cis*-8, *cis*-11, *cis*-14, *cis*-17; 22:5 *cis*-7, *cis*-10, *cis*-13, *cis*-16, *cis*-19; 22:6 *cis*-4, *cis*-7, *cis*-10, *cis*-13, *cis*-16, *cis*-19.

[9] Sum of n-6 fatty acids, consisting of 18:2 *cis*-9, *cis*-12; 18:3 *cis*-6, *cis*-9, *cis*-12; 20:2, *cis*-11, *cis*-14; 20:3 *cis*-8, *cis*-11, *cis*-14; 20:4 *cis*-5, *cis*-8, *cis*-11, *cis*-14; 22:2, *cis*-13, *cis*-16; 22:4 *cis*-7, *cis*-10, *cis*-13, *cis*-16; 22:5 *cis*-4, *cis*-7, *cis*-10, *cis*-13, *cis*-16.

[10] Δ-5 desaturase index = [20:4 *cis*-5, *cis*-8, *cis*-11, *cis*-14] : [20:3 *cis*-8, *cis*-11, *cis*-14 + 20:4 *cis*-5, *cis*-8, *cis*-11, *cis*-14].

[11] Δ-6 desaturase index = [20:3 *cis*-8, *cis*-11, *cis*-14] : [18:2 *cis*-9, *cis*-12 + 20:3 *cis*-8, *cis*-11, *cis*-14].

[12] Δ-9 desaturase index = [18:1 *cis*-9] : [18:0 + 18:1 *cis*-9].

**Supplemental Table S2.2: Effects of the maternal supplementation with coconut oil (CTRL; n = 9), linseed and safflower oil (EFA; n = 9), Lutalin (CLA; *cis*-9, *trans*-11 and *trans*-10, *cis*-12; n = 9), or a combination of the EFA and CLA supplement (EFA+CLA; n = 11) on the fatty acid concentration in blood plasma of calves on d 1 and 5 of life[1]**

| | | Maternal supplementation | | | | *P*-value[2] | | |
|---|---|---|---|---|---|---|---|---|
| Fatty acid, µg/mL plasma[3] | Time[4] | CTRL | EFA | CLA | EFA+CLA | EFA<br>Time | CLA<br>EFA × time | EFA × CLA<br>CLA × time |
| 10:0 | 1 | 0.75 ± 0.51 | 0.92 ± 0.47$^{B}$ | 0.77 ± 0.49$^{B}$ | 0.93 ± 0.40 | 0.91 | 0.99 | 0.42 |
| | 5 | 3.18 ± 0.84 | 3.57 ± 0.81$^{A}$ | 3.82 ± 0.82$^{A}$ | 2.89 ± 0.72 | <0.001 | 0.57 | 0.97 |
| 11:0 | 1 | 0.05 ± 0.05 | 0.02 ± 0.04$^{B*}$ | 0.06 ± 0.05 | 0.07 ± 0.04 | 0.64 | 0.44 | 0.44 |
| | 5 | 0.21 ± 0.07 | 0.27 ± 0.06$^{A}$ | 0.20 ± 0.06 | 0.11 ± 0.06 | <0.001 | 0.97 | 0.05 |
| 12:0 | 1 | 2.21 ± 1.25$^{B}$ | 1.28 ± 1.15$^{B}$ | 1.24 ± 1.21$^{B}$ | 1.35 ± 0.99$^{B}$ | 0.15 | 0.25 | 0.97 |
| | 5 | 12.97 ± 2.12$^{A}$ | 10.92 ± 2.07$^{A}$ | 11.64 ± 2.10$^{A}$ | 8.39 ± 1.84$^{A}$ | <0.001 | 0.28 | 0.48 |
| 13:0 | 1 | 0.09 ± 0.05$^{B}$ | 0.04 ± 0.05$^{B*}$ | 0.09 ± 0.05$^{B}$ | 0.03 ± 0.04$^{B*}$ | 0.06 | 0.26 | 0.39 |
| | 5 | 0.49 ± 0.08$^{A}$ | 0.45 ± 0.08$^{A}$ | 0.47 ± 0.08$^{A}$ | 0.31 ± 0.07$^{A}$ | <0.001 | 0.56 | 0.37 |
| 14:0 | 1 | 12.73 ± 6.85$^{B}$ | 6.00 ± 6.33$^{B}$ | 7.71 ± 6.64$^{B}$ | 6.60 ± 5.48$^{B}$ | 0.19 | 0.80 | 0.78 |
| | 5 | 63.91 ± 10.37$^{A}$ | 59.57 ± 10.03$^{A}$ | 69.12 ± 10.23$^{A}$ | 53.77 ± 8.92$^{A}$ | <0.001 | 0.57 | 0.85 |
| 14:1 *cis*-9 | 1 | 1.86 ± 0.81 | 1.06 ± 0.76 | 1.66 ± 0.79 | 0.97 ± 0.67 | 0.08 | 0.22 | 0.65 |
| | 5 | 4.00 ± 0.76 | 2.81 ± 0.71 | 2.77 ± 0.74 | 2.24 ± 0.62 | <0.001 | 0.88 | 0.33 |
| 15:0 | 1 | 3.51 ± 0.66$^{B}$ | 3.31 ± 0.61$^{B}$ | 2.56 ± 0.64$^{B}$ | 2.82 ± 0.53$^{B}$ | 0.23 | 0.23 | 0.70 |
| | 5 | 8.50 ± 0.90$^{A}$ | 7.96 ± 0.86$^{A}$ | 8.76 ± 0.88$^{A}$ | 7.12 ± 0.77$^{A}$ | <0.001 | 0.23 | 0.64 |
| 16:0 | 1 | 109 ± 31$^{B}$ | 109 ± 28$^{B}$ | 100 ± 30$^{B}$ | 113 ± 25$^{B}$ | 0.69 | 0.66 | 0.89 |
| | 5 | 345 ± 43$^{A}$ | 334 ± 42$^{A}$ | 342 ± 43$^{A}$ | 307 ± 37$^{A}$ | <0.001 | 0.52 | 0.79 |
| 16:1*cis*-9 | 1 | 18.7 ± 4.3$^{B}$ | 21.9 ± 3.9 | 19.0 ± 4.1$^{B}$ | 19.4 ± 3.4 | 0.04 | 0.25 | 0.51 |
| | 5 | 50.9 ± 5.8$^{a,A}$ | 38.9 ± 5.6$^{ab}$ | 47.9 ± 5.8$^{ab,A}$ | 31.3 ± 5.0$^{b}$ | <0.001 | <0.01 | 0.49 |

**Supplemental Table S2.2: Continuation**

| Fatty acid, µg/mL plasma[3] | Time[4] | Maternal supplementation | | | | *P*-value[2] | | |
|---|---|---|---|---|---|---|---|---|
| | | CTRL | EFA | CLA | EFA+CLA | EFA<br>Time | CLA<br>EFA × time | EFA × CLA<br>CLA × time |
| 17:0 | 1 | 4.63 ± 0.97$^{B}$ | 5.51 ± 0.90$^{B}$ | 4.48 ± 0.94$^{B}$ | 5.46 ± 0.78$^{B}$ | 0.88 | 0.72 | 0.98 |
| | 5 | 12.77 ± 1.35$^{A}$ | 11.72 ± 1.30$^{A}$ | 12.48 ± 1.33$^{A}$ | 11.27 ± 1.15$^{A}$ | <0.001 | 0.13 | 0.84 |
| 17:1*cis*-9 | 1 | 3.32 ± 0.62$^{B}$ | 4.01 ± 0.58$^{B}$ | 3.28 ± 0.60$^{B}$ | 3.95 ± 0.50 | 0.02 | 0.15 | 0.83 |
| | 5 | 10.06 ± 0.86$^{a,A}$ | 7.07 ± 0.83$^{b,A}$ | 8.76 ± 0.85$^{ab,A}$ | 6.14 ± 0.74$^{b}$ | <0.001 | <0.001 | 0.24 |
| 18:0 | 1 | 42.3 ± 10.8$^{B}$ | 53.7 ± 9.9$^{B}$ | 44.9 ± 10.4$^{B}$ | 52.7 ± 8.6$^{B}$ | 0.47 | 0.46 | 0.49 |
| | 5 | 165.6 ± 17.6$^{A}$ | 178.2 ± 17.1$^{A}$ | 186.7 ± 17.4$^{A}$ | 180.0 ± 15.2$^{A}$ | <0.001 | 0.70 | 0.54 |
| 18:1*cis*-9 | 1 | 88.8 ± 23.4$^{B}$ | 114.6 ± 21.6$^{B}$ | 91.8 ± 22.7$^{B}$ | 107.4 ± 18.7$^{B}$ | 0.19 | 0.28 | 0.95 |
| | 5 | 320.4 ± 32.4$^{A}$ | 248.9 ± 31.1$^{A}$ | 281.4 ± 31.9$^{A}$ | 223.7 ± 27.6$^{A}$ | <0.001 | <0.01 | 0.34 |
| 18:1*cis*-11 | 1 | 14.5 ± 2.7$^{B}$ | 19.4 ± 2.5 | 16.7 ± 2.6 | 17.8 ± 2.2 | 0.94 | 0.17 | 0.55 |
| | 5 | 28.4 ± 3.3$^{A}$ | 25.0 ± 3.1 | 23.9 ± 3.2 | 20.8 ± 2.8 | <0.001 | 0.10 | 0.23 |
| 18:1*trans*-9 | 1 | 0.23 ± 0.19$^{B}$ | 0.23 ± 0.17$^{B}$ | 0.48 ± 0.18$^{B}$ | 0.34 ± 0.15$^{B}$ | 0.03 | 0.18 | 0.11 |
| | 5 | 4.27 ± 0.57$^{ab,A}$ | 3.87 ± 0.57$^{ab,A}$ | 5.76 ± 0.57$^{a,A}$ | 3.60 ± 0.51$^{b,A}$ | <0.001 | 0.03 | 0.43 |
| 18:1*trans*-11 | 1 | 0.24 ± 0.21$^{B}$ | 0.14 ± 0.19$^{B}$ | 0.05 ± 0.20$^{B}$ | 0.11 ± 0.16$^{B}$ | 0.55 | 0.77 | 0.90 |
| | 5 | 7.72 ± 0.96$^{A}$ | 7.13 ± 0.96$^{A}$ | 7.51 ± 0.96$^{A}$ | 6.99 ± 0.87$^{A}$ | <0.001 | 0.56 | 0.95 |
| 18:2 *cis*-9, *cis*-12 | 1 | 14.8 ± 8.5$^{B}$ | 26.0 ± 7.8$^{B}$ | 17.9 ± 8.2$^{B}$ | 29.1 ± 6.7$^{B}$ | 0.34 | 0.70 | 0.82 |
| (LA) | 5 | 314.4 ± 29.0$^{A}$ | 324.9 ± 28.8$^{A}$ | 315.7 ± 28.9$^{A}$ | 340.0 ± 25.9$^{A}$ | <0.001 | 0.82 | 0.85 |
| 18:2 *cis*-9, *trans*-11 | 1 | 0.27 ± 0.15$^{B}$ | 0.28 ± 0.14$^{B}$ | 0.36 ± 0.15$^{B}$ | 0.43 ± 0.12$^{B}$ | 0.13 | <0.001 | 0.89 |
| CLA | 5 | 3.11 ± 0.48$^{bc,A}$ | 2.30 ± 0.48$^{c,A}$ | 5.27 ± 0.48$^{a,A}$ | 4.53 ± 0.43$^{ab,A}$ | <0.001 | 0.09 | <0.001 |
| 18:2 *trans*-10, *cis*-12 | 1 | 0.00 ± 0.10$^{*}$ | 0.05 ± 0.09 | 0.00 ± 0.09$^{B*}$ | 0.06 ± 0.08$^{B}$ | 0.53 | <0.001 | 0.93 |
| CLA | 5 | 0.00 ± 0.24$^{b*}$ | 0.02 ± 0.24$^{b*}$ | 2.16 ± 0.24$^{a,A}$ | 2.25 ± 0.21$^{a,A}$ | <0.001 | 0.96 | <0.001 |
| 18:3 *cis*-6, *cis*-9, *cis*-12 | 1 | 1.52 ± 0.31$^{B}$ | 1.77 ± 0.28$^{B}$ | 1.36 ± 0.30$^{B}$ | 1.80 ± 0.24 | <0.01 | 0.31 | 0.51 |
| | 5 | 5.20 ± 0.52$^{a,A}$ | 3.54 ± 0.51$^{ab,A}$ | 5.16 ± 0.52$^{a,A}$ | 2.62 ± 0.45$^{b}$ | <0.001 | <0.001 | 0.38 |
| 18:3 *cis*-9, *cis*-12, *cis*-15 | 1 | 0.00 ± 1.13$^{b*}$ | 2.31 ± 1.03$^{ab,B}$ | 0.40 ± 1.09$^{ab}$ | 3.00 ± 0.89$^{a,B}$ | <0.001 | 0.80 | 0.89 |
| (ALA) | 5 | 20.90 ± 8.41$^{b}$ | 114.47 ± 8.40$^{a,A}$ | 21.49 ± 8.40$^{b}$ | 116.96 ± 7.59$^{a,A}$ | <0.001 | <0.001 | 0.90 |

**Supplemental Table S2.2: Continuation**

| Fatty acid, µg/mL plasma[3] | Time[4] | Maternal supplementation | | | | *P*-value[2] | | |
|---|---|---|---|---|---|---|---|---|
| | | CTRL | EFA | CLA | EFA+CLA | EFA<br>Time | CLA<br>EFA × time | EFA × CLA<br>CLA × time |
| 18:4 *cis*-6, *cis*-9, | 1 | 0.06 ± 0.10$^{B}$ | 0.00 ± 0.09$^{B*}$ | 0.08 ± 0.09$^{B}$ | 0.08 ± 0.08 | 0.16 | 0.54 | 0.48 |
| *cis*-12, *cis*-15 | 5 | 0.85 ± 0.16$^{A}$ | 0.82 ± 0.16$^{A}$ | 0.84 ± 0.16$^{A}$ | 0.48 ± 0.14 | <0.001 | 0.30 | 0.08 |
| 20:0 | 1 | 1.61 ± 0.16 | 1.62 ± 0.15 | 1.46 ± 0.16$^{B}$ | 1.57 ± 0.14 | 0.79 | 0.88 | 0.86 |
| | 5 | 1.95 ± 0.15 | 1.93 ± 0.14 | 2.16 ± 0.15$^{A}$ | 1.98 ± 0.12 | <0.001 | 0.33 | 0.18 |
| 20:1*cis*-11 | 1 | 0.45 ± 0.14$^{B}$ | 0.61 ± 0.13$^{B}$ | 0.45 ± 0.14$^{B}$ | 0.63 ± 0.11$^{B}$ | 0.87 | 0.40 | 0.35 |
| | 5 | 2.30 ± 0.27$^{A}$ | 1.87 ± 0.26$^{A}$ | 1.85 ± 0.27$^{A}$ | 1.88 ± 0.24$^{A}$ | <0.001 | 0.18 | 0.39 |
| 20:2, *cis*-11, *cis*-14 | 1 | 0.56 ± 0.17$^{B}$ | 0.62 ± 0.16$^{B}$ | 0.67 ± 0.16$^{B}$ | 0.82 ± 0.14$^{B}$ | 0.51 | <0.01 | 0.61 |
| | 5 | 1.61 ± 0.22$^{b,A}$ | 1.51 ± 0.21$^{b,A}$ | 2.37 ± 0.22$^{a,A}$ | 1.96 ± 0.19$^{ab,A}$ | <0.001 | 0.11 | 0.05 |
| 20:3, *cis*-8, *cis*-11, | 1 | 12.2 ± 2.4$^{B}$ | 15.5 ± 2.3 | 11.0 ± 2.4 | 16.1 ± 2.0 | 0.88 | 0.22 | 0.74 |
| *cis*-14 | 5 | 21.3 ± 2.2$^{a,A}$ | 17.7 ± 2.1$^{ab}$ | 18.8 ± 2.2$^{ab}$ | 14.9 ± 1.8$^{b}$ | <0.001 | <0.01 | 0.34 |
| 20:3, *cis*-11, *cis*-14, | 1 | 0.00 ± 0.08$^{b*}$ | 0.11 ± 0.07$^{ab,B}$ | 0.01 ± 0.08$^{b*}$ | 0.23 ± 0.06$^{a,B}$ | <0.001 | 0.04 | 0.06 |
| *cis*-17 | 5 | 0.14 ± 0.17$^{b}$ | 1.26 ± 0.17$^{a,A}$ | 0.11 ± 0.17$^{b}$ | 1.77 ± 0.15$^{a,A}$ | <0.001 | <0.001 | 0.42 |
| 20:4 *cis*-5, *cis*-8, *cis*-11, | 1 | 30.2 ± 5.4$^{B}$ | 26.6 ± 5.1$^{B}$ | 29.5 ± 5.3$^{B}$ | 30.0 ± 4.5 | 0.01 | 0.48 | 0.44 |
| *cis*-14 (ARA) | 5 | 62.5 ± 5.2$^{a,A}$ | 47.0 ± 4.9$^{b,A}$ | 55.3 ± 5.0$^{ab,A}$ | 43.9 ± 4.3$^{b}$ | <0.001 | 0.05 | 0.29 |
| 20:5 *cis*-5, *cis*-8, *cis*-11, | 1 | 1.69 ± 0.90$^{b,B}$ | 6.29 ± 0.82$^{a,B}$ | 1.97 ± 0.87$^{b}$ | 7.05 ± 0.71$^{a,B}$ | <0.001 | 0.81 | 0.78 |
| *cis*-14, *cis*-17 (EPA) | 5 | 7.98 ± 1.87$^{b,A}$ | 21.49 ± 1.83$^{a,A}$ | 7.76 ± 1.85$^{b}$ | 19.84 ± 1.64$^{a,A}$ | <0.001 | <0.001 | 0.44 |
| 21:0 | 1 | 0.71 ± 0.05 | 0.73 ± 0.04 | 0.67 ± 0.04 | 0.67 ± 0.04 | 0.11 | 0.64 | 0.06 |
| | 5 | 0.59 ± 0.04$^{b}$ | 0.73 ± 0.04$^{a}$ | 0.73 ± 0.04$^{a}$ | 0.72 ± 0.03$^{a}$ | 0.90 | 0.24 | 0.02 |
| 22:0 | 1 | 2.91 ± 0.25 | 3.08 ± 0.24 | 2.81 ± 0.24 | 3.00 ± 0.21 | 0.42 | 0.81 | 0.94 |
| | 5 | 2.73 ± 0.17 | 2.75 ± 0.16 | 2.74 ± 0.16 | 2.78 ± 0.13 | 0.07 | 0.48 | 0.59 |
| 22:1, *cis*-13 | 1 | 0.03 ± 0.02$^{*}$ | 0.01 ± 0.02$^{*}$ | 0.01 ± 0.02$^{B*}$ | 0.01 ± 0.01$^{*}$ | 0.09 | 0.87 | 0.42 |
| | 5 | 0.19 ± 0.05 | 0.16 ± 0.05 | 0.26 ± 0.05$^{A}$ | 0.12 ± 0.05 | <0.001 | 0.18 | 0.67 |
| 22:2, *cis*-13, *cis*-16 | 1 | 0.00 ± 0.39$^{*}$ | 0.14 ± 0.37 | 0.00 ± 0.38$^{*}$ | 0.23 ± 0.32 | 0.09 | 0.89 | 0.21 |
| | 5 | 0.00 ± 0.40$^{*}$ | 0.63 ± 0.37 | 0.22 ± 0.39 | 0.06 ± 0.33 | 0.24 | 0.75 | 0.60 |

**Supplemental Table S2.2: Continuation**

| Fatty acid, µg/mL plasma[3] | Time[4] | Maternal supplementation | | | | *P*-value[2] | | |
|---|---|---|---|---|---|---|---|---|
| | | CTRL | EFA | CLA | EFA+CLA | EFA<br>Time | CLA<br>EFA × time | EFA × CLA<br>CLA × time |
| 22:4 *cis*-7, *cis*-10, *cis*- | 1 | 1.92 ± 0.37$^{B}$ | 1.18 ± 0.34 | 2.18 ± 0.36$^{B}$ | 1.24 ± 0.30 | <0.001 | 0.60 | 0.48 |
| 13, *cis*-16 | 5 | 3.60 ± 0.40$^{a,A}$ | 2.09 ± 0.38$^{b}$ | 3.87 ± 0.39$^{a,A}$ | 1.95 ± 0.33$^{b}$ | <0.001 | 0.02 | 0.78 |
| 22:5 *cis*-4, *cis*-7, *cis*-10, | 1 | 0.49 ± 0.19 | 0.30 ± 0.18 | 0.64 ± 0.18$^{B}$ | 0.18 ± 0.15 | <0.001 | 0.92 | 0.12 |
| *cis*-13, *cis*-16 | 5 | 1.07 ± 0.20$^{ab}$ | 0.61 ± 0.18$^{bc}$ | 1.24 ± 0.19$^{a,A}$ | 0.38 ± 0.16$^{c}$ | <0.001 | 0.06 | 0.80 |
| 22:5 *cis*-7, *cis*-10, *cis*-13, | 1 | 4.83 ± 1.13$^{ab}$ | 7.84 ± 1.07$^{ab,B}$ | 4.57 ± 1.11$^{b}$ | 8.05 ± 0.94$^{a,B}$ | <0.001 | 0.85 | 0.67 |
| *cis*-16, *cis*-19 (DPA) | 5 | 7.36 ± 1.15$^{b}$ | 12.83 ± 1.09$^{a,A}$ | 6.92 ± 1.12$^{b}$ | 12.89 ± 0.96$^{a,A}$ | <0.001 | 0.07 | 0.90 |
| 22:6 *cis*-4, *cis*-7, *cis*-10, | 1 | 4.55 ± 1.34$^{b}$ | 8.96 ± 1.25$^{a}$ | 4.72 ± 1.31$^{b}$ | 8.60 ± 1.09$^{a}$ | <0.001 | 0.72 | 0.78 |
| *cis*-13, *cis*-16, *cis*-19 (DHA) | 5 | 5.79 ± 1.35$^{ab}$ | 9.54 ± 1.25$^{a}$ | 5.49 ± 1.31$^{b}$ | 8.95 ± 1.09$^{ab}$ | 0.22 | 0.65 | 0.77 |
| 23:0 | 1 | 1.28 ± 0.37 | 0.82 ± 0.34 | 0.65 ± 0.36 | 0.57 ± 0.30 | 0.24 | 0.73 | 0.99 |
| | 5 | 0.77 ± 0.52 | 0.60 ± 0.50 | 1.23 ± 0.52 | 0.69 ± 0.45 | 0.97 | 0.88 | 0.16 |
| 24:0 | 1 | 3.03 ± 0.25 | 3.22 ± 0.24 | 2.85 ± 0.25 | 3.13 ± 0.21 | 0.40 | 0.73 | 0.77 |
| | 5 | 2.78 ± 0.21 | 2.76 ± 0.19 | 2.80 ± 0.20 | 2.83 ± 0.17 | 0.02 | 0.30 | 0.42 |
| SFA[5] | 1 | 185 ± 49$^{B}$ | 188 ± 45$^{B}$ | 170 ± 47$^{B}$ | 191 ± 39$^{B}$ | 0.74 | 0.85 | 0.76 |
| | 5 | 621 ± 72$^{A}$ | 615 ± 70$^{A}$ | 644 ± 71$^{A}$ | 580 ± 62$^{A}$ | <0.001 | 0.52 | 1.0 |
| MUFA[6] | 1 | 128 ± 32$^{B}$ | 162 ± 29$^{B}$ | 133 ± 31$^{B}$ | 151 ± 25$^{B}$ | 0.16 | 0.26 | 0.94 |
| | 5 | 428 ± 43$^{A}$ | 336 ± 41$^{A}$ | 380 ± 42$^{A}$ | 297 ± 37$^{A}$ | <0.001 | 0.01 | 0.34 |
| PUFA[7] | 1 | 72.0 ± 20.8$^{B}$ | 103.0 ± 19.0$^{B}$ | 77.8 ± 20.1$^{B}$ | 108.2 ± 16.4$^{B}$ | <0.01 | 0.82 | 0.93 |
| | 5 | 455.4 ± 45.5$^{A}$ | 566.2 ± 44.7$^{A}$ | 456.4 ± 45.1$^{A}$ | 574.7 ± 40.1$^{A}$ | <0.001 | 0.06 | 0.99 |
| Sum of n-3 fatty acids[8] | 1 | 10.9 ± 4.1$^{b}$ | 26.4 ± 3.7$^{a,B}$ | 12.4 ± 3.9$^{b}$ | 27.1 ± 3.2$^{a,B}$ | <0.001 | 0.92 | 0.94 |
| | 5 | 42.9 ± 11.6$^{b}$ | 161.4 ± 11.5$^{a,A}$ | 43.2 ± 11.5$^{b}$ | 161.0 ± 10.3$^{a,A}$ | <0.001 | <0.001 | 0.93 |
| Sum of n-6 fatty acids[9] | 1 | 60.0 ± 16.8$^{B}$ | 76.1 ± 15.4$^{B}$ | 65.0 ± 16.2$^{B}$ | 80.2 ± 13.2$^{B}$ | 0.71 | 0.89 | 0.92 |
| | 5 | 408.1 ± 35.5$^{A}$ | 401.9 ± 34.9$^{A}$ | 404.5 ± 35.3$^{A}$ | 406.4 ± 31.2$^{A}$ | <0.001 | 0.59 | 0.90 |
| Ratio n-6 : n-3 fatty acids | 1 | 5.18 ± 0.31$^{a,B}$ | 2.97 ± 0.28$^{b}$ | 5.52 ± 0.30$^{a,B}$ | 2.91 ± 0.24$^{b}$ | <0.001 | 0.55 | 0.36 |
| | 5 | 9.42 ± 0.41$^{a,A}$ | 2.70 ± 0.39$^{b}$ | 9.73 ± 0.40$^{a,A}$ | 2.62 ± 0.34$^{b}$ | <0.001 | <0.001 | 0.94 |

[a-c] LSM within a row with different lowercase letters differ between treatments ($P < 0.05$).

[A, B] LSM within a column with different uppercase letters differ between days of life ($P < 0.05$).

[*] LSM were below the detection limit of 0.01%.

[1] Values are presented as LSM ± SE.

[2] *P*-values for fixed effects are presented in 2 rows: The first row indicates *P*-values for the effect of EFA, CLA, and their interaction; the second row indicates *P*-values for the effect of time and interactions between EFA or CLA and time.

[3] Concentration of the presented fatty acid in plasma.

[4] Day of life.

[5] Sum of saturated fatty acids, consisting of 10:0; 11:0; 12:0; 13:0; 14:0; 15:0; 16:0; 17:0; 18:0; 20:0; 21:0; 22:0; 23:0; 24:0.

[6] Sum of monounsaturated fatty acids, consisting of 14:1 *cis*-9; 16:1 *cis*-9; 17:1 *cis*-9; 18:1 *cis*-9; 18:1 *cis*-11; 18:1 *trans*-9; 18:1 *trans*-11; 20:1 *cis*-11; 22:1 *cis*-13.

[7] Sum of polyunsaturated fatty acids, consisting of 18:2 *cis*-9, *cis*-12; 18:2 *cis*-9, *trans*-11; 18:2 *trans*-10, *cis*-12; 18:2 *trans*-9, *trans*-12; 18:3 *cis*-6, *cis*-9, *cis*-12; 18:3 *cis*-9, *cis*-12, *cis*-15; 18:4 *cis*-6, *cis*-9, *cis*-12, *cis*-15; 20:2 *cis*-11, *cis*-14; 20:3 *cis*-8, *cis*-11, *cis*-14; 20:3 *cis*-11, *cis*-14, *cis*-17; 20:4 *cis*-5, *cis*-8, *cis*-11, *cis*-14; 20:5 *cis*-5, *cis*-8, *cis*-11, *cis*-14, *cis*-17; 22:2 *cis*-13, *cis*-16; 22:4 *cis*-7, *cis*-10, *cis*-13, *cis*-16; 22:5 *cis*-4, *cis*-7, *cis*-10, *cis*-13, *cis*-16; 22:5 *cis*-7, *cis*-10, *cis*-13, *cis*-16, *cis*-19; 22:6 *cis*-4, *cis*-7, *cis*-10, *cis*-13, *cis*-16, *cis*-19.

[8] Sum of n-3 fatty acids, consisting of 18:3 *cis*-9, *cis*-12, *cis*-15; 18:4 *cis*-6, *cis*-9, *cis*-12, *cis*-15; 20:3, *cis*-11, *cis*-14, *cis*-17; 20:5 *cis*-5, *cis*-8, *cis*-11, *cis*-14, *cis*-17; 22:5 *cis*-7, *cis*-10, *cis*-13, *cis*-16, *cis*-19; 22:6 *cis*-4, *cis*-7, *cis*-10, *cis*-13, *cis*-16, *cis*-19.

[9] Sum of n-6 fatty acids, consisting of 18:2 *cis*-9, *cis*-12; 18:3 *cis*-6, *cis*-9, *cis*-12; 20:2, *cis*-11, *cis*-14; 20:3 *cis*-8, *cis*-11, *cis*-14; 20:4 *cis*-5, *cis*-8, *cis*-11, *cis*-14; 22:2, *cis*-13, *cis*-16; 22:4 *cis*-7, *cis*-10, *cis*-13, *cis*-16; 22:5 *cis*-4, *cis*-7, *cis*-10, *cis*-13, *cis*-16.

**Supplemental Table S2.3: Effects of supplementing coconut oil (CTRL, n = 9), linseed and safflower oil (EFA, n = 9), Lutalin (CLA, *cis*-9, *trans*-11 and *trans*-10, *cis*-12, n = 10), or a combination of EFA and CLA (EFA+CLA, n = 10) on the fatty acid composition in maternal plasma and colostrum[1]**

| | | Maternal supplementation | | | |
|---|---|---|---|---|---|
| Fatty acid, %[2] | Item[3] | CTRL | EFA | CLA | EFA+CLA |
| ALA | Plasma | 1.93 ± 0.41$^{b}$ | 8.62 ± 0.41$^{a}$ | 1.94 ± 0.43$^{b}$ | 9.14 ± 0.39$^{a}$ |
| | Colostrum | 0.07 ± 0.11$^{b}$ | 1.34 ± 0.10$^{a}$ | 0.09 ± 0.10$^{b}$ | 1.55 ± 0.10$^{a}$ |
| EPA | Plasma | 0.34 ± 0.08$^{b}$ | 0.99 ± 0.07$^{a}$ | 0.36 ± 0.08$^{b}$ | 0.78 ± 0.07$^{a}$ |
| | Colostrum | 0.03 ± 0.01$^{b}$ | 0.10 ± 0.01$^{a}$ | 0.03 ± 0.01$^{b}$ | 0.10 ± 0.01$^{a}$ |
| DPA | Plasma | 0.63 ± 0.06$^{c}$ | 1.43 ± 0.06$^{a}$ | 0.70 ± 0.06$^{c}$ | 1.19 ± 0.05$^{b}$ |
| | Colostrum | 0.12 ± 0.01$^{c}$ | 0.21 ± 0.01$^{b}$ | 0.11 ± 0.01$^{c}$ | 0.28 ± 0.01$^{a}$ |
| DHA | Plasma | 0.13 ± 0.01$^{bc}$ | 0.23 ± 0.01$^{a}$ | 0.12 ± 0.02$^{c}$ | 0.18 ± 0.01$^{ab}$ |
| | Colostrum | -$^{*}$ | - | - | - |
| LA | Plasma | 32.39 ± 1.08 | 31.27 ± 1.07 | 31.19 ± 1.14 | 31.88 ± 1.01 |
| | Colostrum | 2.63 ± 0.16$^{b}$ | 2.99 ± 0.16$^{ab}$ | 2.40 ± 0.16$^{b}$ | 3.27 ± 0.15$^{a}$ |
| ARA | Plasma | 2.93 ± 0.12$^{a}$ | 2.33 ± 0.11$^{b}$ | 2.81 ± 0.12$^{a}$ | 2.24 ± 0.11$^{b}$ |
| | Colostrum | 0.26 ± 0.02$^{a}$ | 0.17 ± 0.02$^{b}$ | 0.23 ± 0.02$^{a}$ | 0.20 ± 0.02$^{ab}$ |
| c-9, t-11 CLA | Plasma | 0.21 ± 0.02$^{b}$ | 0.14 ± 0.02$^{b}$ | 0.47 ± 0.02$^{a}$ | 0.41 ± 0.02$^{a}$ |
| | Colostrum | 0.27 ± 0.06 | 0.23 ± 0.05 | 0.33 ± 0.05 | 0.33 ± 0.05 |
| t-10, c-12 CLA | Plasma | 0.00 ± 0.01$^{b§}$ | 0.00 ± 0.01$^{§*}$ | 0.15 ± 0.01$^{a}$ | 0.16 ± 0.01$^{a}$ |
| | Colostrum | 0.10 ± 0.03 | 0.11 ± 0.03 | 0.17 ± 0.03 | 0.21 ± 0.03 |

[a-c] LSM within a row with different lowercase letters differ between treatments ($P < 0.05$).

[*] Not detected in colostrum.

[§] LSM were below the detection limit of 0.01%.

APPENDIX

[1] Values are presented as LSM ± SE in % of total fatty acids; Data of FA in blood plasma was obtained from Gnott et al. (2020). Data FA in colostrum was obtained from Vogel et al. (2020). Data were analyzed using the MIXED procedure of SAS by repeated measures ANOVA. The model included the fixed factors EFA (level: yes, no), CLA (level: yes, no), time (levels: d relative to calving), block (levels: 1 to 5), and the respective interactions (EFA × CLA; EFA × time; CLA × time; EFA × CLA × time) and calving interval as well as milk yield in 2nd lactation as covariates. The Tukey-Kramer test was applied to test differences between LSM.

[2] Proportion of the presented fatty acid in total plasma or colostrum fatty acids.

[3] Plasma was sampled on d 1 postpartum, colostrum was from first milking postpartum.

**Supplemental Table S2.4: Effects of the maternal supplementation with coconut oil (CTRL; n = 9), linseed and safflower oil (EFA; n = 9), Lutalin (CLA; *cis*-9, *trans*-11 and *trans*-10, *cis*-12; n = 9), or a combination of the EFA and CLA supplement (EFA+CLA; n = 11) on the fatty acid composition in plasma triglycerides of calves on d 1 and 5 of life[1]**

| | | Maternal supplementation | | | | *P*-value[2] | | |
|---|---|---|---|---|---|---|---|---|
| Fatty acid, %[3] | Time[4] | CTRL | EFA | CLA | EFA+CLA | EFA<br>Time | CLA<br>EFA × time | EFA × CLA<br>CLA × time |
| 10:0 | 1 | 1.40 ±0.82 | 1.41 ±0.77 | 1.87 ±0.80 | 1.79 ±0.67 | 0.98 | 0.85 | 0.67 |
| | 5 | 1.25 ±0.68 | 0.83 ±0.64 | 0.57 ±0.66 | 1.02 ±0.54 | 0.01 | 0.93 | 0.22 |
| 11:0 | 1 | 0.19 ±0.05 | 0.18 ±0.04 | 0.22 ±0.05 | 0.20 ±0.04 | 0.41 | 0.37 | 0.37 |
| | 5 | 0.09 ±0.10 | 0.11 ±0.10 | 0.25 ±0.10 | 0.09 ±0.08 | 0.17 | 0.57 | 0.63 |
| 12:0 | 1 | 3.89 ±1.39 | 3.19 ±1.33 | 1.76 ±1.36 | 1.50 ±1.17 | 0.82 | 0.11 | 0.65 |
| | 5 | 1.14 ±1.02 | 1.52 ±0.96 | 0.22 ±0.98 | 1.49 ±0.80 | 0.01 | 0.26 | 0.21 |
| 13:0 | 1 | 0.19 ±0.05 | 0.23 ±0.04 | 0.18 ±0.05 | 0.18 ±0.04 | 0.54 | 0.22 | 0.51 |
| | 5 | 0.11 ±0.03 | 0.13 ±0.02 | 0.09 ±0.02 | 0.09 ±0.02 | <0.001 | 0.71 | 0.93 |
| 14:0 | 1 | 4.52 ±0.99 | 3.52 ±0.91$^{B}$ | 4.45 ±0.96$^{B}$ | 4.47 ±0.79$^{B}$ | 0.63 | 0.46 | 0.44 |
| | 5 | 7.35 ±1.16 | 8.68 ±1.14$^{A}$ | 9.31 ±1.13$^{A}$ | 7.70 ±0.96$^{A}$ | <0.001 | 0.71 | 0.96 |
| 14:1 *cis*-9 | 1 | 0.28 ±0.18 | 0.11 ±0.18 | 0.09 ±0.18 | 0.31 ±0.16 | 0.94 | 0.64 | 0.18 |
| | 5 | 0.32 ±0.11 | 0.22 ±0.10 | 0.16 ±0.10 | 0.19 ±0.08 | 0.73 | 0.68 | 0.51 |
| 15:0 | 1 | 1.86 ±0.36 | 2.27 ±0.35 | 2.36 ±0.36$^{A}$ | 2.31 ±0.31$^{A}$ | 0.55 | 0.56 | 0.39 |
| | 5 | 1.27 ±0.24 | 1.46 ±0.22 | 1.36 ±0.23$^{B}$ | 1.31 ±0.19$^{B}$ | <0.001 | 0.66 | 0.27 |
| 16:0 | 1 | 35.1 ±2.5 | 34.4 ±2.3 | 35.8 ±2.4 | 36.6 ±2.0 | 0.96 | 0.40 | 0.62 |
| | 5 | 36.6 ±2.5 | 38.8 ±2.4 | 39.7 ±2.4 | 37.6 ±2.0 | 0.02 | 0.99 | 0.77 |
| 16:1 *cis*-9 | 1 | 2.39 ±1.32 | 4.50 ±1.31 | 1.80 ±1.32 | 1.33 ±1.18 | 0.51 | 0.08 | 0.23 |
| | 5 | 2.42 ±0.38 | 2.73 ±0.36 | 2.29 ±0.37 | 2.07 ±0.30 | 0.85 | 0.55 | 0.26 |

**Supplemental Table S2.4: Continuation**

| Fatty acid, %[3] | Time[4] | Maternal supplementation | | | | *P*-value[2] | | |
|---|---|---|---|---|---|---|---|---|
| | | CTRL | EFA | CLA | EFA+CLA | EFA<br>Time | CLA<br>EFA × time | EFA × CLA<br>CLA × time |
| 17:0 | 1 | 2.10 ±0.22 | 2.46 ±0.21 | 2.45 ±0.22 | 2.60 ±0.19$^{A}$ | 0.14 | 0.14 | 0.64 |
| | 5 | 1.82 ±0.15 | 1.93 ±0.14 | 1.93 ±0.14 | 2.03 ±0.12$^{B}$ | <0.001 | 0.42 | 0.50 |
| 17:1 *cis*-9 | 1 | 0.90 ±0.84 | 0.33 ±0.77 | 0.06 ±0.81 | 1.04 ±0.66 | 0.72 | 0.43 | 0.14 |
| | 5 | 1.89 ±0.92 | 0.41 ±0.87 | 0.23 ±0.89 | 0.47 ±0.74 | 0.48 | 0.08 | 0.12 |
| 18:0 | 1 | 17.1 ±1.9$^{A}$ | 14.8 ±1.8 | 16.3 ±1.9 | 16.3 ±1.6 | 0.62 | 0.37 | 0.30 |
| | 5 | 12.1 ±1.6$^{B}$ | 11.3 ±1.5 | 12.6 ±1.5 | 13.8 ±1.2 | <0.001 | 0.36 | 0.45 |
| 18:1 *cis*-9 | 1 | 4.42 ±2.49$^{B}$ | 6.28 ±2.29$^{B}$ | 5.44 ±2.41$^{B}$ | 2.80 ±1.98$^{B}$ | 0.54 | 0.25 | 0.41 |
| | 5 | 17.85 ±2.79$^{A}$ | 16.53 ±2.70$^{A}$ | 15.81 ±2.71$^{A}$ | 13.90 ±2.28$^{A}$ | <0.001 | 0.56 | 0.59 |
| 18:1 *cis*-11 | 1 | 0.70 ±0.27 | 1.05 ±0.26 | 0.92 ±0.26 | 0.56 ±0.23 | 0.60 | 0.21 | 0.21 |
| | 5 | 1.17 ±0.19 | 1.02 ±0.18 | 0.95 ±0.18 | 0.83 ±0.15 | 0.13 | 0.60 | 0.77 |
| 18:1 *trans*-9 | 1 | 0.10 ±0.04 | 0.09 ±0.04 | 0.08 ±0.04$^{B}$ | 0.08 ±0.03$^{B}$ | 0.79 | 0.08 | 0.75 |
| | 5 | 0.29 ±0.05 | 0.29 ±0.05 | 0.39 ±0.05$^{A}$ | 0.36 ±0.05$^{A}$ | <0.001 | 0.78 | 0.13 |
| 18:1 *trans*-11 | 1 | 0.36 ±0.14 | 0.12 ±0.13 | 0.19 ±0.13 | 0.13 ±0.12$^{B}$ | 0.15 | 0.53 | 0.54 |
| | 5 | 0.62 ±0.09 | 0.60 ±0.08 | 0.65 ±0.08 | 0.59 ±0.07$^{A}$ | <0.001 | 0.44 | 0.54 |
| 18:2 *cis*-9, *cis*-12 | 1 | 0.84 ±0.42$^{B}$ | 1.07 ±0.38 | 1.43 ±0.40 | 0.62 ±0.33$^{B}$ | 0.59 | 0.57 | 0.56 |
| (LA) | 5 | 2.96 ±0.56$^{A}$ | 2.74 ±0.56 | 2.38 ±0.55 | 2.55 ±0.47$^{A}$ | <0.001 | 0.62 | 0.39 |
| 18:2 *cis*-9, *trans*-11 | 1 | 0.00 ±0.08$^{*}$ | 0.02 ±0.08 | 0.09 ±0.08 | 0.00 ±0.07$^{*}$ | 0.33 | 0.19 | 0.11 |
| CLA | 5 | 0.08 ±0.04$^{b}$ | 0.09 ±0.04$^{b}$ | 0.20 ±0.04$^{a}$ | 0.11 ±0.03$^{ab}$ | <0.01 | 0.95 | 0.61 |
| 18:2 *trans*-9, *trans*-12 | 1 | 0.47 ±0.13 | 0.24 ±0.12 | 0.47 ±0.13 | 0.50 ±0.11 | 0.68 | 0.15 | 0.97 |
| | 5 | 0.16 ±0.11 | 0.33 ±0.10 | 0.35 ±0.10 | 0.27 ±0.09 | 0.02 | 0.20 | 0.62 |
| 18:3 *cis*-6, *cis*-9, *cis*-12 | 1 | 0.76 ±0.22 | 0.89 ±0.22 | 0.84 ±0.22 | 0.94 ±0.19 | 0.34 | 0.87 | 0.89 |
| | 5 | 0.31 ±0.09 | 0.43 ±0.09 | 0.30 ±0.09 | 0.38 ±0.07 | <0.001 | 0.94 | 0.66 |
| 18:3 *cis*-9, *cis*-12, *cis*-15 | 1 | 0.06 ±0.04 | 0.11 ±0.04$^{B}$ | 0.11 ±0.04 | 0.05 ±0.04$^{B}$ | <0.001 | 0.72 | 0.62 |
| (ALA) | 5 | 0.27 ±0.18$^{b}$ | 1.52 ±0.19$^{a,A}$ | 0.24 ±0.18$^{b}$ | 1.41 ±0.17$^{a,A}$ | <0.001 | <0.001 | 0.66 |

**Supplemental Table S2.4: Continuation**

| Fatty acid, %[3] | Time[4] | Maternal supplementation | | | | *P*-value[2] | | |
|---|---|---|---|---|---|---|---|---|
| | | CTRL | EFA | CLA | EFA+CLA | EFA<br>Time | CLA<br>EFA × time | EFA × CLA<br>CLA × time |
| 18:4 *cis*-6, *cis*-9, | 1 | 0.00 ±0.02$^{*}$ | 0.00 ±0.02$^{*}$ | 0.03 ±0.02 | 0.00 ±0.02$^{*}$ | 0.36 | 0.02 | 0.55 |
| *cis*-12, *cis*-15 | 5 | 0.00 ±0.02$^{*}$ | 0.00 ±0.02$^{*}$ | 0.04 ±0.02 | 0.04 ±0.02 | 0.08 | 0.31 | 0.17 |
| 20:0 | 1 | 1.86 ±0.49 | 2.37 ±0.48 | 2.79 ±0.49$^{A}$ | 1.99 ±0.43 | 0.94 | 0.71 | 0.25 |
| | 5 | 0.94 ±0.26 | 1.00 ±0.25 | 0.81 ±0.25$^{B}$ | 0.97 ±0.21 | <0.001 | 0.55 | 0.43 |
| 20:1*cis*-11 | 1 | 0.26 ±0.12 | 0.06 ±0.12 | 0.06 ±0.12 | 0.15 ±0.11 | 0.57 | 0.57 | 0.20 |
| | 5 | 0.16 ±0.04 | 0.13 ±0.03 | 0.13 ±0.03 | 0.13 ±0.03 | 0.94 | 0.70 | 0.73 |
| 20:2 *cis*-11, *cis*-14 | 1 | 0.00 ±0.36$^{*}$ | 0.00 ±0.36$^{*}$ | 0.00 ±0.36$^{*}$ | 0.58 ±0.33 | 0.41 | 0.39 | 0.41 |
| | 5 | 0.02 ±0.22 | 0.03 ±0.22 | 0.03 ±0.22 | 0.41 ±0.20 | 0.80 | 0.57 | 0.48 |
| 20:3 *cis*-5, *cis*-8, | 1 | 0.07 ±0.04 | 0.10 ±0.04 | 0.16 ±0.04 | 0.06 ±0.03 | 0.28 | 0.64 | 0.08 |
| *cis*-11 | 5 | 0.09 ±0.01 | 0.09 ±0.01 | 0.09 ±0.01 | 0.07 ±0.01 | 0.42 | 0.40 | 0.34 |
| 20:3 *cis*-8, *cis*-11, | 1 | 0.67 ±0.24 | 1.20 ±0.23$^{A}$ | 0.75 ±0.24 | 0.76 ±0.21 | 0.22 | 0.33 | 0.42 |
| *cis*-14 | 5 | 0.20 ±0.11 | 0.16 ±0.11$^{B}$ | 0.09 ±0.11 | 0.18 ±0.09 | <0.001 | 0.28 | 0.55 |
| 20:3 *cis*-11, *cis*-14, | 1 | 0.01 ±0.01 | 0.00 ±0.01$^{B*}$ | 0.00 ±0.01$^{*}$ | 0.00 ±0.01$^{B*}$ | <0.01 | 0.77 | 0.18 |
| *cis*-17 | 5 | 0.00 ±0.01$^{b*}$ | 0.04 ±0.01$^{ab,A}$ | 0.00 ±0.01$^{b*}$ | 0.05 ±0.01$^{a,A}$ | <0.001 | <0.001 | 0.44 |
| 20:4 *cis*-5, *cis*-8, *cis*-11, | 1 | 1.13 ±0.43 | 1.76 ±0.41$^{A}$ | 1.14 ±0.42$^{A}$ | 0.54 ±0.37 | 0.72 | 0.13 | 0.29 |
| *cis*-14 (ARA) | 5 | 0.11 ±0.27 | 0.10 ±0.25$^{B}$ | 0.00 ±0.26$^{B*}$ | 0.18 ±0.21 | <0.001 | 0.74 | 0.17 |
| 20:5 *cis*-5, *cis*-8, *cis*-11, | 1 | 0.01 ±0.00 | 0.00 ±0.00$^{*}$ | 0.00 ±0.00$^{*}$ | 0.00 ±0.00$^{B*}$ | 0.05 | 0.52 | 0.10 |
| *cis*-14, *cis*-17 (EPA) | 5 | 0.02 ±0.02 | 0.04 ±0.02 | 0.01 ±0.02 | 0.09 ±0.02$^{A}$ | <0.01 | 0.04 | 0.38 |
| 21:0 | 1 | 0.90 ±0.18$^{A}$ | 1.07 ±0.17$^{A}$ | 0.89 ±0.18 | 1.08 ±0.15$^{A}$ | 0.23 | 0.59 | 0.91 |
| | 5 | 0.38 ±0.13$^{B}$ | 0.44 ±0.12$^{B}$ | 0.47 ±0.12 | 0.56 ±0.10$^{B}$ | <0.001 | 0.43 | 0.42 |
| 22:0 | 1 | 4.57 ±0.59$^{A}$ | 4.30 ±0.56$^{A}$ | 4.05 ±0.58$^{A}$ | 4.31 ±0.50$^{A}$ | 1.00 | 0.51 | 0.55 |
| | 5 | 2.13 ±0.44$^{B}$ | 2.00 ±0.41$^{B}$ | 1.81 ±0.43$^{B}$ | 1.95 ±0.35$^{B}$ | <0.001 | 0.98 | 0.87 |

**Supplemental Table S2.4: Continuation**

| Fatty acid, %[3] | Time[4] | Maternal supplementation | | | | *P*-value[2] | | |
|---|---|---|---|---|---|---|---|---|
| | | CTRL | EFA | CLA | EFA+CLA | EFA<br>Time | CLA<br>EFA × time | EFA × CLA<br>CLA × time |
| 22:1 *cis*-13 | 1 | 0.00 ±0.00$^{*}$ | 0.00 ±0.00$^{*}$ | 0.00 ±0.00$^{*}$ | 0.00 ±0.00$^{*}$ | 0.44 | 0.60 | 0.07 |
| | 5 | 0.00 ±0.00$^{*}$ | 0.00 ±0.00$^{*}$ | 0.00 ±0.00$^{*}$ | 0.00 ±0.00$^{*}$ | 0.93 | 0.52 | 0.55 |
| 22:2 *cis*-13, *cis*-16 | 1 | 0.65 ±0.40 | 0.76 ±0.37 | 0.93 ±0.39 | 1.03 ±0.33 | 0.43 | 0.24 | 0.88 |
| | 5 | 0.19 ±0.34 | 0.54 ±0.31 | 0.52 ±0.32 | 0.74 ±0.26 | <0.01 | 0.43 | 0.96 |
| 22:4 *cis*-7, *cis*-10, *cis*- | 1 | 0.08 ±0.05 | 0.00 ±0.04$^{*}$ | 0.00 ±0.04$^{*}$ | 0.00 ±0.04$^{*}$ | <0.05 | 0.09 | 0.08 |
| 13, *cis*-16 | 5 | 0.10 ±0.03$^{a}$ | 0.00 ±0.03$^{b*}$ | 0.02 ±0.03$^{ab}$ | 0.00 ±0.03$^{b*}$ | 0.63 | 0.76 | 0.84 |
| 22:5 *cis*-4, *cis*-7, *cis*-10, | 1 | 0.01 ±0.03 | 0.00 ±0.03$^{*}$ | 0.00 ±0.03$^{*}$ | 0.01 ±0.02 | 0.70 | 0.85 | 0.31 |
| *cis*-13, *cis*-16 | 5 | 0.00 ±0.01$^{*}$ | 0.00 ±0.01$^{*}$ | 0.00 ±0.01$^{*}$ | 0.00 ±0.01$^{*}$ | 0.34 | 0.67 | 0.86 |
| 22:5 *cis*-7, *cis*-10, *cis*-13, | 1 | 4.28 ±0.79$^{A}$ | 4.30 ±0.76$^{A}$ | 4.10 ±0.78$^{A}$ | 5.46 ±0.67$^{A}$ | 0.31 | 0.32 | 0.33 |
| *cis*-16, *cis*-19 (DPA) | 5 | 1.77 ±0.59$^{B}$ | 1.82 ±0.55$^{B}$ | 1.97 ±0.57$^{B}$ | 2.42 ±0.46$^{B}$ | <0.001 | 0.43 | 0.87 |
| 22:6 *cis*-4, *cis*-7, *cis*-10, | 1 | 0.26 ±0.16 | 0.46 ±0.16 | 0.48 ±0.16 | 0.26 ±0.14 | 0.94 | 0.95 | 0.26 |
| *cis*-13, *cis*-16, *cis*-19 (DHA) | 5 | 0.11 ±0.05 | 0.10 ±0.05 | 0.06 ±0.05 | 0.11 ±0.04 | <0.001 | 0.84 | 0.86 |
| 23:0 | 1 | 1.46 ±0.52 | 2.06 ±0.51 | 1.55 ±0.51 | 2.37 ±0.45 | 0.07 | 0.67 | 0.43 |
| | 5 | 0.56 ±0.38 | 1.15 ±0.37 | 1.00 ±0.37 | 0.68 ±0.31 | <0.01 | 0.37 | 0.73 |
| 24:0 | 1 | 5.17 ±0.90$^{A}$ | 5.58 ±0.86$^{A}$ | 5.36 ±0.88$^{A}$ | 6.65 ±0.76$^{A}$ | 0.23 | 0.36 | 0.58 |
| | 5 | 2.12 ±0.65$^{B}$ | 2.43 ±0.60$^{B}$ | 2.30 ±0.62$^{B}$ | 2.86 ±0.51$^{B}$ | <0.001 | 0.51 | 0.61 |
| SFA[5] | 1 | 82.2 ±2.6$^{A}$ | 77.6 ±2.5 | 81.3 ±2.6 | 83.1 ±2.2$^{A}$ | 0.67 | <0.05 | 0.40 |
| | 5 | 69.8 ±2.4$^{B}$ | 71.1 ±2.3 | 73.7 ±2.3 | 72.9 ±1.9$^{B}$ | <0.001 | 0.57 | 0.86 |
| MUFA[6] | 1 | 8.99 ±3.41$^{B}$ | 12.01 ±3.19$^{B}$ | 8.48 ±3.32$^{B}$ | 6.48 ±2.79$^{B}$ | 0.63 | 0.10 | 0.60 |
| | 5 | 24.29 ±3.06$^{A}$ | 21.31 ±2.90$^{A}$ | 20.46 ±2.95$^{A}$ | 18.60 ±2.43$^{A}$ | <0.001 | 0.29 | 0.93 |
| PUFA[7] | 1 | 8.57 ±1.71 | 10.23 ±1.60 | 9.88 ±1.66$^{A}$ | 10.25 ±1.40 | 0.11 | 0.55 | 0.97 |
| | 5 | 5.68 ±1.48 | 7.29 ±1.39 | 5.55 ±1.43$^{B}$ | 8.34 ±1.17 | <0.001 | 0.35 | 0.87 |

**Supplemental Table S2.4: Continuation**

| Fatty acid, %[3] | Time[4] | Maternal supplementation | | | | *P*-value[2] | | |
|---|---|---|---|---|---|---|---|---|
| | | CTRL | EFA | CLA | EFA+CLA | EFA<br>Time | CLA<br>EFA × time | EFA × CLA<br>CLA × time |
| Sum of n-3 fatty acids[8] | 1 | 4.36 ±0.73$^{A}$ | 4.61 ±0.70 | 4.37 ±0.72$^{A}$ | 5.57 ±0.62$^{A}$ | <0.01 | 0.35 | 0.38 |
| | 5 | 1.93 ±0.52$^{b,B}$ | 3.30 ±0.49$^{ab}$ | 1.96 ±0.50$^{b,B}$ | 3.74 ±0.41$^{a,B}$ | <0.001 | 0.16 | 0.68 |
| Sum of n-6 fatty acids[9] | 1 | 3.85 ±0.98 | 5.27 ±0.91 | 4.86 ±0.95 | 4.16 ±0.79 | 0.46 | 0.97 | 0.68 |
| | 5 | 3.58 ±1.01 | 3.54 ±0.97 | 3.01 ±0.98 | 4.14 ±0.82 | 0.02 | 0.82 | 0.93 |
| Ratio n-6 : n-3 fatty acids | 1 | 1.00 ±0.59$^{ab,B}$ | 2.07 ±0.54$^{a}$ | 1.21 ±0.57$^{ab}$ | 0.62 ±0.47$^{b}$ | 0.01 | 0.12 | 0.38 |
| | 5 | 4.47 ±0.82$^{a,A}$ | 1.76 ±0.82$^{ab}$ | 3.68 ±0.80$^{ab}$ | 1.18 ±0.69$^{b}$ | <0.001 | <0.001 | 0.94 |

$^{a-c}$ LSM within a row with different lowercase letters differ between treatments ($P < 0.05$).

$^{A, B}$ LSM within a column with different uppercase letters differ between days of life ($P < 0.05$).

$^{*}$ LSM were below the detection limit of 0.01%.

[1] Values are presented as LSM ± SE.

[2] *P*-values for fixed effects are presented in 2 rows: The first row indicates *P*-values for the effect of EFA, CLA, and their interaction; the second row indicates *P*-values for the effect of time and interactions between EFA or CLA and time.

[3] Proportion of the presented fatty acid in total triglycerides.

[4] Day of life.

[5] Sum of saturated fatty acids, consisting of 10:0; 11:0; 12:0; 13:0; 14:0; 15:0; 16:0; 17:0; 18:0; 20:0; 21:0; 22:0; 23:0; 24:0.

[6] Sum of monounsaturated fatty acids, consisting of 14:1 *cis*-9; 16:1 *cis*-9; 17:1 *cis*-9; 18:1 *cis*-9; 18:1 *cis*-11; 18:1 *trans*-9; 18:1 *trans*-11; 20:1 *cis*-11; 22:1 *cis*-13.

[7] Sum of polyunsaturated fatty acids, consisting of 18:2 *cis*-9, *cis*-12; 18:2 *cis*-9, *trans*-11; 18:2 *trans*-9, *trans*-12; 18:3 *cis*-6, *cis*-9, *cis*-12; 18:3 *cis*-9, *cis*-12, *cis*-15; 18:4 *cis*-6, *cis*-9, *cis*-12, *cis*-15; 20:2 *cis*-11, *cis*-14; 20:3 *cis*-5, *cis*-8, *cis*-11; 20:3 *cis*-8, *cis*-11, *cis*-14; 20:3 *cis*-11, *cis*-14, *cis*-17; 20:4 *cis*-5, *cis*-8, *cis*-11, *cis*-14; 20:5 *cis*-5, *cis*-8, *cis*-11, *cis*-14, *cis*-17; 22:2 *cis*-13, *cis*-16; 22:4 *cis*-7, *cis*-10, *cis*-13, *cis*-16; 22:5 *cis*-4, *cis*-7, *cis*-10, *cis*-13, *cis*-16; 22:5 *cis*-7, *cis*-10, *cis*-13, *cis*-16, *cis*-19; 22:6 *cis*-4, *cis*-7, *cis*-10, *cis*-13, *cis*-16, *cis*-19.

[8] Sum of n-3 fatty acids, consisting of 18:3 *cis*-9, *cis*-12, *cis*-15; 18:4 *cis*-6, *cis*-9, *cis*-12, *cis*-15; 20:3 *cis*-11, *cis*-14, *cis*-17; 20:5 *cis*-5, *cis*-8, *cis*-11, *cis*-14, *cis*-17; 22:5 *cis*-7, *cis*-10, *cis*-13, *cis*-16, *cis*-19; 22:6 *cis*-4, *cis*-7, *cis*-10, *cis*-13, *cis*-16, *cis*-19.

[9] Sum of n-6 fatty acids, consisting of 18:2 *cis*-9, *cis*-12; 18:3 *cis*-6, *cis*-9, *cis*-12; 20:2 *cis*-11, *cis*-14; 20:3 *cis*-8, *cis*-11, *cis*-14; 20:4 *cis*-5, *cis*-8, *cis*-11, *cis*-14; 22:2 *cis*-13, *cis*-16; 22:4 *cis*-7, *cis*-10, *cis*-13, *cis*-16; 22:5 *cis*-4, *cis*-7, *cis*-10, *cis*-13, *cis*-16.

**Supplemental Table S2.5: Effects of the maternal supplementation with coconut oil (CTRL; n = 9), linseed and safflower oil (EFA; n = 9), Lutalin (CLA; *cis*-9, *trans*-11 and *trans*-10, *cis*-12; n = 9), or a combination of the EFA and CLA supplement (EFA+CLA; n = 11) on the fatty acid composition in plasma phospholipids of calves on d 1 and 5 of life[1]**

| | | Maternal supplementation | | | | *P*-value[2] | | |
|---|---|---|---|---|---|---|---|---|
| Fatty acid, %[3] | Time[4] | CTRL | EFA | CLA | EFA+CLA | EFA<br>Time | CLA<br>EFA × time | EFA × CLA<br>CLA × time |
| 10:0 | 1 | 0.42 ± 0.16 | 0.33 ± 0.15 | 0.20 ± 0.15 | 0.18 ± 0.13 | 0.27 | 0.12 | 0.33 |
| | 5 | 0.23 ± 0.15 | 0.00 ± 0.14$^{*}$ | 0.02 ± 0.14 | 0.00 ± 0.12$^{*}$ | <0.01 | 0.55 | 0.44 |
| 11:0 | 1 | 0.04 ± 0.08 | 0.07 ± 0.08 | 0.06 ± 0.08 | 0.14 ± 0.07 | 0.68 | 0.68 | 0.82 |
| | 5 | 0.03 ± 0.03 | 0.01 ± 0.03 | 0.02 ± 0.03 | 0.00 ± 0.02$^{*}$ | 0.09 | 0.33 | 0.43 |
| 12:0 | 1 | 1.17 ± 0.50 | 1.72 ± 0.50 | 1.21 ± 0.50 | 0.56 ± 0.45 | 0.96 | 0.21 | 0.16 |
| | 5 | 0.13 ± 0.17 | 0.28 ± 0.16 | 0.18 ± 0.16 | 0.07 ± 0.13 | <0.001 | 0.87 | 0.31 |
| 13:0 | 1 | 0.08 ± 0.04 | 0.07 ± 0.03 | 0.07 ± 0.03 | 0.09 ± 0.03 | 0.59 | 0.65 | 0.16 |
| | 5 | 0.03 ± 0.04 | 0.00 ± 0.04$^{*}$ | 0.00 ± 0.04$^{*}$ | 0.06 ± 0.03 | <0.001 | 0.85 | 0.85 |
| 14:0 | 1 | 1.94 ± 0.30$^{A}$ | 1.56 ± 0.30 | 1.89 ± 0.30$^{A}$ | 1.86 ± 0.27$^{A}$ | 0.35 | 0.67 | 0.32 |
| | 5 | 0.95 ± 0.12$^{B}$ | 0.77 ± 0.11 | 0.84 ± 0.11$^{B}$ | 0.88 ± 0.09$^{B}$ | <0.001 | 0.66 | 0.70 |
| 14:1 *cis*-9 | 1 | 0.22 ± 0.08 | 0.29 ± 0.08$^{A}$ | 0.15 ± 0.08 | 0.21 ± 0.07 | 0.32 | 0.46 | 0.98 |
| | 5 | 0.04 ± 0.03 | 0.06 ± 0.02$^{B}$ | 0.05 ± 0.03 | 0.07 ± 0.02 | <0.001 | 0.55 | 0.17 |
| 15:0 | 1 | 1.01 ± 0.18 | 1.01 ± 0.18$^{A}$ | 1.23 ± 0.18$^{A}$ | 1.14 ± 0.16$^{A}$ | 0.84 | 0.34 | 0.87 |
| | 5 | 0.43 ± 0.05 | 0.42 ± 0.05$^{B}$ | 0.41 ± 0.05$^{B}$ | 0.42 ± 0.04$^{B}$ | <0.001 | 0.83 | 0.30 |
| 16:0 | 1 | 26.6 ± 1.3$^{A}$ | 24.6 ± 1.3$^{A}$ | 25.8 ± 1.3$^{A}$ | 26.7 ± 1.1$^{A}$ | 0.60 | 0.60 | 0.10 |
| | 5 | 21.6 ± 0.8$^{B}$ | 20.6 ± 0.7$^{B}$ | 20.9 ± 0.8$^{B}$ | 21.5 ± 0.6$^{B}$ | <0.001 | 0.73 | 0.61 |
| 16:1 *cis*-9 | 1 | 1.69 ± 0.16$^{a}$ | 1.15 ± 0.15$^{b}$ | 1.23 ± 0.15$^{ab}$ | 1.12 ± 0.13$^{b}$ | <0.001 | 0.07 | 0.11 |
| | 5 | 1.25 ± 0.11$^{a}$ | 0.89 ± 0.11$^{bc}$ | 1.20 ± 0.11$^{ab}$ | 0.89 ± 0.09$^{c}$ | <0.01 | 0.92 | 0.18 |

**Supplemental Table S2.5: Continuation**

| Fatty acid, %[3] | Time[4] | Maternal supplementation | | | | *P*-value[2] | | |
|---|---|---|---|---|---|---|---|---|
| | | CTRL | EFA | CLA | EFA+CLA | EFA<br>Time | CLA<br>EFA × time | EFA × CLA<br>CLA × time |
| 17:0 | 1 | 2.31 ± 0.20$^{ab,A}$ | 2.45 ± 0.20$^{ab,A}$ | 1.98 ± 0.20$^{b}$ | 2.71 ± 0.18$^{a,A}$ | 0.03 | 0.94 | 0.08 |
| | 5 | 1.49 ± 0.09$^{B}$ | 1.44 ± 0.08$^{B}$ | 1.44 ± 0.09 | 1.51 ± 0.07$^{B}$ | <0.001 | 0.03 | 0.81 |
| 17:1 *cis*-9 | 1 | 0.43 ± 0.12 | 0.42 ± 0.11 | 0.44 ± 0.12 | 0.38 ± 0.10 | 0.21 | 0.21 | 0.69 |
| | 5 | 0.95 ± 0.23 | 0.52 ± 0.24 | 0.51 ± 0.23 | 0.34 ± 0.20 | 0.10 | 0.18 | 0.13 |
| 18:0 | 1 | 23.3 ± 0.9 | 22.9 ± 0.8 | 23.1 ± 0.9 | 22.7 ± 0.7 | 0.33 | 0.23 | 0.73 |
| | 5 | 21.0 ± 0.9$^{b}$ | 22.8 ± 0.9$^{ab}$ | 22.8 ± 0.9$^{ab}$ | 23.9 ± 0.7$^{a}$ | 0.34 | 0.01 | 0.02 |
| 18:1 *cis*-9 | 1 | 12.6 ± 1.1$^{B}$ | 13.2 ± 1.0 | 13.2 ± 1.1$^{B}$ | 12.5 ± 0.9 | <0.001 | 0.55 | 0.42 |
| | 5 | 19.5 ± 1.1$^{a,A}$ | 15.0 ± 1.0$^{b}$ | 19.2 ± 1.1$^{a,A}$ | 14.1 ± 0.9$^{b}$ | <0.001 | <0.001 | 0.60 |
| 18:1 *cis*-11 | 1 | 2.89 ± 0.24$^{A}$ | 3.26 ± 0.23$^{A}$ | 3.05 ± 0.24$^{A}$ | 2.88 ± 0.21$^{A}$ | 0.76 | 0.16 | 0.32 |
| | 5 | 2.01 ± 0.16$^{a,B}$ | 1.81 ± 0.16$^{ab,B}$ | 1.76 ± 0.16$^{ab,B}$ | 1.60 ± 0.13$^{b,B}$ | <0.001 | 0.22 | 0.61 |
| 18:1 *trans*-9 | 1 | 0.12 ± 0.05 | 0.09 ± 0.04 | 0.08 ± 0.05$^{B}$ | 0.14 ± 0.04 | 0.23 | 0.17 | 0.78 |
| | 5 | 0.17 ± 0.04$^{ab}$ | 0.11 ± 0.04$^{b}$ | 0.26 ± 0.04$^{a,A}$ | 0.15 ± 0.03$^{b}$ | <0.001 | <0.01 | 0.09 |
| 18:1 *trans*-11 | 1 | 0.09 ± 0.04$^{B}$ | 0.04 ± 0.04$^{B}$ | 0.02 ± 0.04$^{B}$ | 0.07 ± 0.03$^{B}$ | 0.78 | 0.02 | 0.05 |
| | 5 | 0.60 ± 0.04$^{A}$ | 0.57 ± 0.04$^{A}$ | 0.52 ± 0.04$^{A}$ | 0.53 ± 0.03$^{A}$ | <0.001 | 0.84 | 0.56 |
| 18:2 *cis*-9, *cis*-12 | 1 | 2.34 ± 0.45$^{B}$ | 3.02 ± 0.41$^{B}$ | 2.55 ± 0.44$^{B}$ | 2.78 ± 0.36$^{B}$ | 0.02 | 0.86 | 0.47 |
| (LA) | 5 | 17.03 ± 1.07$^{A}$ | 19.81 ± 1.11$^{A}$ | 17.78 ± 1.06$^{A}$ | 19.47 ± 0.94$^{A}$ | <0.001 | 0.10 | 0.83 |
| 18:2 *cis*-9, *trans*-11 | 1 | 0.02 ± 0.02$^{B}$ | 0.03 ± 0.02$^{B}$ | 0.03 ± 0.02$^{B}$ | 0.01 ± 0.02$^{B}$ | 0.16 | <0.001 | 0.04 |
| CLA | 5 | 0.11 ± 0.02$^{b,A}$ | 0.11 ± 0.02$^{b,A}$ | 0.24 ± 0.02$^{a,A}$ | 0.19 ± 0.01$^{a,A}$ | <0.001 | 0.44 | <0.001 |
| 18:2 *trans*-9, *trans*-12 | 1 | 0.25 ± 0.09 | 0.21 ± 0.09 | 0.34 ± 0.09 | 0.32 ± 0.08 | 0.55 | 0.29 | 0.93 |
| | 5 | 0.14 ± 0.02 | 0.12 ± 0.02 | 0.15 ± 0.02 | 0.12 ± 0.02 | <0.01 | 0.91 | 0.31 |
| 18:3 *cis*-6, *cis*-9, *cis*-12 | 1 | 0.22 ± 0.07 | 0.27 ± 0.07 | 0.21 ± 0.07 | 0.30 ± 0.07$^{A}$ | 0.76 | 0.80 | 0.86 |
| | 5 | 0.12 ± 0.02$^{a}$ | 0.05 ± 0.02$^{b}$ | 0.14 ± 0.02$^{a}$ | 0.04 ± 0.02$^{b,B}$ | <0.001 | <0.05 | 0.99 |
| 18:3 *cis*-9, *cis*-12, *cis*-15 | 1 | 0.08 ± 0.02 | 0.12 ± 0.01$^{B}$ | 0.13 ± 0.02 | 0.13 ± 0.01$^{B}$ | <0.001 | 0.52 | 0.23 |
| (ALA) | 5 | 0.59 ± 0.16$^{b}$ | 3.33 ± 0.17$^{a,A}$ | 0.63 ± 0.16$^{b}$ | 3.02 ± 0.15$^{a,A}$ | <0.001 | <0.001 | 0.33 |

**Supplemental Table S2.5: Continuation**

| Fatty acid, %[3] | Time[4] | Maternal supplementation | | | | *P*-value[2] | | |
|---|---|---|---|---|---|---|---|---|
| | | CTRL | EFA | CLA | EFA+CLA | EFA<br>Time | CLA<br>EFA × time | EFA × CLA<br>CLA × time |
| 18:4 *cis*-6, *cis*-9, | 1 | 0.17 ± 0.11 | 0.07 ± 0.11 | 0.06 ± 0.11 | 0.22 ± 0.10 | 0.90 | 0.88 | 0.26 |
| *cis*-12, *cis*-15 | 5 | 0.02 ± 0.01$^{ab}$ | 0.01 ± 0.01$^{ab}$ | 0.03 ± 0.01$^{a}$ | 0.01 ± 0.01$^{b}$ | 0.04 | 0.70 | 0.90 |
| 20:0 | 1 | 0.99 ± 0.10$^{A}$ | 0.85 ± 0.10$^{A}$ | 0.99 ± 0.10$^{A}$ | 0.86 ± 0.09$^{A}$ | 0.12 | 0.80 | 0.68 |
| | 5 | 0.28 ± 0.06$^{B}$ | 0.22 ± 0.05$^{B}$ | 0.27 ± 0.05$^{B}$ | 0.28 ± 0.04$^{B}$ | <0.001 | 0.25 | 0.83 |
| 20:1 *cis*-11 | 1 | 0.21 ± 0.04 | 0.27 ± 0.04$^{A}$ | 0.23 ± 0.04 | 0.19 ± 0.03 | 0.89 | 0.50 | 0.64 |
| | 5 | 0.20 ± 0.03 | 0.16 ± 0.02$^{B}$ | 0.17 ± 0.03 | 0.18 ± 0.02 | <0.01 | 0.37 | 0.44 |
| 20:2 *cis*-11, *cis*-14 | 1 | 0.14 ± 0.03 | 0.18 ± 0.03 | 0.19 ± 0.03 | 0.20 ± 0.03 | 0.12 | 0.03 | 0.56 |
| | 5 | 0.16 ± 0.03 | 0.18 ± 0.02 | 0.19 ± 0.02 | 0.21 ± 0.02 | 0.95 | 0.86 | 0.87 |
| 20:3 *cis*-5, *cis*-8, | 1 | 0.25 ± 0.06$^{ab}$ | 0.17 ± 0.06$^{b}$ | 0.38 ± 0.06$^{a}$ | 0.11 ± 0.05$^{b}$ | <0.001 | 0.56 | 0.02 |
| *cis*-11 | 5 | 0.20 ± 0.06$^{ab}$ | 0.14 ± 0.05$^{ab}$ | 0.29 ± 0.05$^{a}$ | 0.07 ± 0.04$^{b}$ | 0.03 | 0.37 | 0.56 |
| 20:3 *cis*-8, *cis*-11, | 1 | 5.67 ± 0.74$^{A}$ | 7.07 ± 0.73$^{A}$ | 5.44 ± 0.74$^{A}$ | 6.04 ± 0.66$^{A}$ | 0.65 | 0.25 | 0.54 |
| *cis*-14 | 5 | 3.12 ± 0.26$^{a,B}$ | 2.57 ± 0.25$^{ab,B}$ | 2.92 ± 0.25$^{a,B}$ | 2.19 ± 0.21$^{b,B}$ | <0.001 | 0.01 | 0.60 |
| 20:3 *cis*-11, *cis*-14, | 1 | 0.00 ± 0.03$^{*}$ | 0.00 ± 0.03$^{*}$ | 0.05 ± 0.03 | 0.00 ± 0.02$^{*}$ | 0.40 | 0.37 | 0.70 |
| *cis*-17 | 5 | 0.00 ± 0.02$^{ab*}$ | 0.03 ± 0.02$^{ab}$ | 0.00 ± 0.02$^{b*}$ | 0.04 ± 0.02$^{a}$ | 0.79 | <0.01 | 0.16 |
| 20:4 *cis*-5, *cis*-8, *cis*-11, | 1 | 5.84 ± 0.75 | 4.61 ± 0.74 | 6.19 ± 0.75$^{A}$ | 4.01 ± 0.66 | <0.01 | 0.52 | 0.51 |
| *cis*-14 (ARA) | 5 | 4.47 ± 0.39$^{a}$ | 3.57 ± 0.36$^{ab}$ | 4.14 ± 0.37$^{a,B}$ | 3.09 ± 0.30$^{b}$ | <0.001 | 0.23 | 0.63 |
| 20:5 *cis*-5, *cis*-8, *cis*-11, | 1 | 0.63 ± 0.25 | 0.69 ± 0.25 | 0.41 ± 0.25 | 0.47 ± 0.24 | <0.01 | 0.19 | 0.79 |
| *cis*-14, *cis*-17 (EPA) | 5 | 0.35 ± 0.07$^{b}$ | 1.10 ± 0.07$^{a}$ | 0.31 ± 0.07$^{b}$ | 0.91 ± 0.06$^{a}$ | 0.36 | 0.02 | 0.68 |
| 21:0 | 1 | 0.50 ± 0.07$^{A}$ | 0.43 ± 0.06$^{A}$ | 0.51 ± 0.06$^{A}$ | 0.53 ± 0.06$^{A}$ | 0.94 | 0.03 | 0.36 |
| | 5 | 0.13 ± 0.03$^{b,B}$ | 0.13 ± 0.03$^{b,B}$ | 0.22 ± 0.03$^{a,B}$ | 0.25 ± 0.02$^{a,B}$ | <0.001 | 0.49 | 0.39 |
| 22:0 | 1 | 1.80 ± 0.24$^{A}$ | 1.67 ± 0.24$^{A}$ | 1.84 ± 0.24$^{A}$ | 1.90 ± 0.21$^{A}$ | 0.87 | 0.43 | 0.70 |
| | 5 | 0.50 ± 0.12$^{B}$ | 0.57 ± 0.11$^{B}$ | 0.56 ± 0.11$^{B}$ | 0.64 ± 0.09$^{B}$ | <0.001 | 0.56 | 0.73 |

**Supplemental Table S2.5: Continuation**

| Fatty acid, %[3] | Time[4] | Maternal supplementation | | | | *P*-value[2] | | |
|---|---|---|---|---|---|---|---|---|
| | | CTRL | EFA | CLA | EFA+CLA | EFA<br>Time | CLA<br>EFA × time | EFA × CLA<br>CLA × time |
| 22:1 *cis*-13 | 1 | 0.02 ± 0.01 | 0.01 ± 0.01 | 0.02 ± 0.01 | 0.00 ± 0.01$^{B*}$ | 0.88 | 0.65 | 0.71 |
| | 5 | 0.02 ± 0.01 | 0.04 ± 0.01 | 0.03 ± 0.01 | 0.05 ± 0.01$^{A}$ | <0.001 | 0.01 | 0.43 |
| 22:2 *cis*-13, *cis*-16 | 1 | 0.19 ± 0.14 | 0.42 ± 0.14 | 0.33 ± 0.14 | 0.60 ± 0.12$^{B}$ | <0.05 | 0.19 | 0.91 |
| | 5 | 0.04 ± 0.05$^{b}$ | 0.12 ± 0.04$^{ab}$ | 0.09 ± 0.05$^{ab}$ | 0.17 ± 0.04$^{a,A}$ | <0.001 | 0.14 | 0.31 |
| 22:4 *cis*-7, *cis*-10, *cis*- | 1 | 0.76 ± 0.11$^{ab}$ | 0.48 ± 0.11$^{b}$ | 0.90 ± 0.11$^{a,A}$ | 0.53 ± 0.10$^{ab}$ | <0.001 | 0.15 | 0.33 |
| 13, *cis*-16 | 5 | 0.40 ± 0.06$^{ab}$ | 0.25 ± 0.06$^{b}$ | 0.52 ± 0.06$^{a,B}$ | 0.25 ± 0.05$^{b}$ | <0.001 | 0.32 | 0.76 |
| 22:5 *cis*-4, *cis*-7, *cis*-10, | 1 | 0.14 ± 0.08 | 0.16 ± 0.08 | 0.26 ± 0.08 | 0.17 ± 0.08 | 0.37 | 0.47 | 0.37 |
| *cis*-13, *cis*-16 | 5 | 0.09 ± 0.03$^{ab}$ | 0.07 ± 0.02$^{ab}$ | 0.11 ± 0.02$^{a}$ | 0.04 ± 0.02$^{b}$ | 0.01 | 0.89 | 0.34 |
| 22:5 *cis*-7, *cis*-10, *cis*-13, | 1 | 2.15 ± 0.32$^{A}$ | 2.68 ± 0.32$^{A}$ | 2.12 ± 0.32$^{A}$ | 2.90 ± 0.29$^{A}$ | <0.001 | 0.80 | 0.67 |
| *cis*-16, *cis*-19 (DPA) | 5 | 0.92 ± 0.14$^{b,B}$ | 1.63 ± 0.13$^{a,B}$ | 0.88 ± 0.13$^{b,B}$ | 1.65 ± 0.11$^{a,B}$ | <0.001 | 0.75 | 0.68 |
| 22:6 *cis*-4, *cis*-7, *cis*-10, | 1 | 0.95 ± 0.20$^{b,A}$ | 1.73 ± 0.20$^{a,A}$ | 1.01 ± 0.20$^{ab,A}$ | 1.29 ± 0.18$^{ab,A}$ | <0.01 | 0.32 | 0.21 |
| *cis*-13, *cis*-16, *cis*-19 (DHA) | 5 | 0.40 ± 0.09$^{b,B}$ | 0.69 ± 0.08$^{a,B}$ | 0.40 ± 0.08$^{b,B}$ | 0.62 ± 0.07$^{a,B}$ | <0.001 | 0.09 | 0.33 |
| 23:0 | 1 | 0.71 ± 0.43 | 0.63 ± 0.42 | 1.09 ± 0.43 | 1.08 ± 0.38 | 0.97 | 0.45 | 0.86 |
| | 5 | 0.14 ± 0.21 | 0.31 ± 0.20 | 0.17 ± 0.20 | 0.12 ± 0.16 | <0.001 | 0.76 | 0.20 |
| 24:0 | 1 | 1.71 ± 0.43 | 2.03 ± 0.42$^{A}$ | 2.13 ± 0.43$^{A}$ | 2.63 ± 0.38$^{A}$ | 0.24 | 0.13 | 0.81 |
| | 5 | 0.67 ± 0.16 | 0.80 ± 0.15$^{B}$ | 0.84 ± 0.15$^{B}$ | 1.02 ± 0.13$^{B}$ | <0.001 | 0.48 | 0.38 |
| SFA[5] | 1 | 62.4 ± 2.5$^{A}$ | 60.2 ± 2.5$^{A}$ | 62.0 ± 2.5$^{A}$ | 62.8 ± 2.2$^{A}$ | 0.81 | 0.32 | 0.40 |
| | 5 | 47.6 ± 1.6$^{B}$ | 48.2 ± 1.5$^{B}$ | 48.5 ± 1.5$^{B}$ | 50.5 ± 1.2$^{B}$ | <0.001 | 0.36 | 0.79 |
| MUFA[6] | 1 | 18.1 ± 1.4$^{B}$ | 18.6 ± 1.3 | 18.3 ± 1.4$^{B}$ | 17.4 ± 1.2 | <0.01 | 0.29 | 0.60 |
| | 5 | 24.6 ± 1.4$^{a,A}$ | 19.0 ± 1.3$^{b}$ | 23.6 ± 1.3$^{a,A}$ | 17.7 ± 1.1$^{b}$ | <0.001 | <0.001 | 0.66 |
| PUFA[7] | 1 | 18.9 ± 1.8$^{B}$ | 21.9 ± 1.7$^{B}$ | 19.8 ± 1.7$^{B}$ | 19.7 ± 1.5$^{B}$ | <0.01 | 0.44 | 0.10 |
| | 5 | 27.2 ± 1.6$^{c,A}$ | 33.8 ± 1.5$^{a,A}$ | 28.0 ± 1.5$^{bc,A}$ | 31.5 ± 1.3$^{ab,A}$ | <0.001 | 0.03 | 0.94 |
| Sum of n-3 fatty acids[8] | 1 | 3.77 ± 0.48$^{ab,A}$ | 5.07 ± 0.46$^{a,B}$ | 3.50 ± 0.47$^{b,A}$ | 4.96 ± 0.41$^{ab}$ | <0.001 | 0.32 | 0.80 |
| | 5 | 2.06 ± 0.35$^{b,B}$ | 6.57 ± 0.33$^{a,A}$ | 1.95 ± 0.34$^{b,B}$ | 6.06 ± 0.28$^{a}$ | 0.42 | <0.001 | 0.76 |

**Supplemental Table S2.5: Continuation**

| Fatty acid, %[3] | Time[4] | Maternal supplementation | | | | *P*-value[2] | | |
|---|---|---|---|---|---|---|---|---|
| | | CTRL | EFA | CLA | EFA+CLA | EFA<br>Time | CLA<br>EFA × time | EFA × CLA<br>CLA × time |
| Sum of n-6 fatty acids[9] | 1 | 14.6 ± 1.6[B] | 16.5 ± 1.5[B] | 15.6 ± 1.6[B] | 14.3 ± 1.3[B] | 0.49 | 0.48 | 0.12 |
| | 5 | 24.7 ± 1.5[A] | 27.0 ± 1.4[A] | 25.4 ± 1.4[A] | 25.1 ± 1.2[A] | <0.001 | 0.64 | 1.00 |
| Ratio n-6:n-3 fatty acids | 1 | 5.05 ± 0.70[a,B] | 3.77 ± 0.64[ab] | 5.32 ± 0.68[a,B] | 3.13 ± 0.56[b,B] | <0.001 | 0.96 | 0.33 |
| | 5 | 11.70 ± 0.73[a,A] | 4.60 ± 0.69[b] | 12.22 ± 0.71[a,A] | 4.34 ± 0.58[b,A] | <0.001 | <0.001 | 0.44 |

[a-c] LSM within a row with different lowercase letters differ between treatments ($P < 0.05$).

[A, B] LSM within a column with different uppercase letters differ between days of life ($P < 0.05$).

[*] LSM were below the detection limit of 0.01%.

[1] Values are presented as LSM ± SE.

[2] *P*-values for fixed effects are presented in 2 rows: The first row indicates *P*-values for the effect of EFA, CLA, and their interaction; the second row indicates *P*-values for the effect of time and interactions between EFA or CLA and time.

[3] Proportion of the presented fatty acid in total phospholipids.

[4] Day of life.

[5] Sum of saturated fatty acids, consisting of 10:0; 11:0; 12:0; 13:0; 14:0; 15:0; 16:0; 17:0; 18:0; 20:0; 21:0; 22:0; 23:0; 24:0.

[6] Sum of monounsaturated fatty acids, consisting of 14:1 *cis*-9; 16:1 *cis*-9; 17:1 *cis*-9; 18:1 *cis*-9; 18:1 *cis*-11; 18:1 *trans*-9; 18:1 *trans*-11; 20:1 *cis*-11; 22:1 *cis*-13.

[7] Sum of polyunsaturated fatty acids, consisting of 18:2 *cis*-9, *cis*-12; 18:2 *cis*-9, *trans*-11; 18:2 *trans*-9, *trans*-12; 18:3 *cis*-6, *cis*-9, *cis*-12; 18:3 *cis*-9, *cis*-12, *cis*-15; 18:4 *cis*-6, *cis*-9, *cis*-12, *cis*-15; 20:2 *cis*-11, *cis*-14; 20:3 *cis*-5, *cis*-8, *cis*-11; 20:3 *cis*-8, *cis*-11, *cis*-14; 20:3 *cis*-11, *cis*-14, *cis*-17; 20:4 *cis*-5, *cis*-8, *cis*-11, *cis*-14; 20:5 *cis*-5, *cis*-8, *cis*-11, *cis*-14, *cis*-17; 22:2 *cis*-13, *cis*-16; 22:4 *cis*-7, *cis*-10, *cis*-13, *cis*-16; 22:5 *cis*-4, *cis*-7, *cis*-10, *cis*-13, *cis*-16; 22:5 *cis*-7, *cis*-10, *cis*-13, *cis*-16, *cis*-19; 22:6 *cis*-4, *cis*-7, *cis*-10, *cis*-13, *cis*-16, *cis*-19.

[8] Sum of n-3 fatty acids, consisting of 18:3 *cis*-9, *cis*-12, *cis*-15; 18:4 *cis*-6, *cis*-9, *cis*-12, *cis*-15; 20:3 *cis*-11, *cis*-14, *cis*-17; 20:5 *cis*-5, *cis*-8, *cis*-11, *cis*-14, *cis*-17; 22:5 *cis*-7, *cis*-10, *cis*-13, *cis*-16, *cis*-19; 22:6 *cis*-4, *cis*-7, *cis*-10, *cis*-13, *cis*-16, *cis*-19.

[9] Sum of n-6 fatty acids, consisting of 18:2 *cis*-9, *cis*-12; 18:3 *cis*-6, *cis*-9, *cis*-12; 20:2 *cis*-11, *cis*-14; 20:3 *cis*-8, *cis*-11, *cis*-14; 20:4 *cis*-5, *cis*-8, *cis*-11, *cis*-14; 22:2 *cis*-13, *cis*-16; 22:4 *cis*-7, *cis*-10, *cis*-13, *cis*-16; 22:5 *cis*-4, *cis*-7, *cis*-10, *cis*-13, *cis*-16.

**Supplemental Table S2.6: Effects of the maternal supplementation with coconut oil (CTRL; n = 9), linseed and safflower oil (EFA; n = 9), Lutalin (CLA; *cis*-9, *trans*-11 and *trans*-10, *cis*-12; n = 9), or a combination of the EFA and CLA supplement (EFA+CLA; n = 11) on the fatty acid composition in plasma cholesterol esters of calves on d 1 and 5 of life[1]**

| Fatty acid, %[3] | Time[4] | Maternal supplementation | | | | *P*-value[2] | | |
|---|---|---|---|---|---|---|---|---|
| | | CTRL | EFA | CLA | EFA+CLA | EFA | CLA | EFA × CLA |
| | | | | | | Time | EFA × time | CLA × time |
| 10:0 | 1 | 4.31 ± 1.79 | 4.62 ± 1.69 | 3.86 ± 1.75 | 4.42 ± 1.49 | 0.84 | 0.67 | 0.91 |
| | 5 | 3.45 ± 1.38 | 3.33 ± 1.28 | 2.80 ± 1.33 | 2.87 ± 1.08 | <0.05 | 0.70 | 0.84 |
| 11:0 | 1 | 1.08 ± 0.42 | 1.37 ± 0.40$^{A}$ | 1.02 ± 0.41 | 0.84 ± 0.35 | 0.97 | 0.73 | 0.56 |
| | 5 | 0.35 ± 0.34 | 0.35 ± 0.31$^{B}$ | 0.52 ± 0.32 | 0.46 ± 0.26 | <0.001 | 0.77 | 0.14 |
| 12:0 | 1 | 2.59 ± 0.99 | 2.21 ± 0.94 | 2.05 ± 0.97 | 1.30 ± 0.83 | 0.23 | 0.84 | 0.58 |
| | 5 | 0.47 ± 0.77 | 0.10 ± 0.72 | 1.40 ± 0.74 | 0.19 ± 0.61 | <0.001 | 0.77 | 0.12 |
| 13:0 | 1 | 2.82 ± 1.15 | 3.91 ± 1.06$^{A}$ | 2.29 ± 1.11 | 2.79 ± 0.93 | 0.66 | 0.89 | 0.82 |
| | 5 | 0.99 ± 1.09 | 0.79 ± 1.03$^{B}$ | 1.64 ± 1.05 | 1.45 ± 0.86 | <0.001 | 0.25 | 0.09 |
| 14:0 | 1 | 3.68 ± 0.30 | 3.43 ± 0.27 | 3.89 ± 0.29 | 4.04 ± 0.24 | 0.03 | 0.35 | 0.19 |
| | 5 | 4.90 ± 0.40 | 3.71 ± 0.40 | 4.54 ± 0.39 | 3.99 ± 0.34 | 0.01 | 0.04 | 0.25 |
| 14:1 *cis*-9 | 1 | 0.29 ± 0.05 | 0.25 ± 0.05 | 0.23 ± 0.05 | 0.23 ± 0.04 | 0.14 | 0.33 | 0.85 |
| | 5 | 0.24 ± 0.03$^{ab}$ | 0.22 ± 0.03$^{ab}$ | 0.26 ± 0.03$^{a}$ | 0.18 ± 0.03$^{b}$ | 0.29 | 0.51 | 0.42 |
| 15:0 | 1 | 1.95 ± 0.29 | 2.17 ± 0.27$^{A}$ | 2.21 ± 0.28 | 2.34 ± 0.24$^{A}$ | 0.86 | 0.17 | 0.95 |
| | 5 | 1.34 ± 0.26 | 1.07 ± 0.25$^{B}$ | 1.53 ± 0.25 | 1.33 ± 0.21$^{B}$ | <0.001 | 0.08 | 0.97 |
| 16:0 | 1 | 28.7 ± 2.1$^{A}$ | 27.5 ± 1.9$^{A}$ | 27.3 ± 2.0$^{A}$ | 29.5 ± 1.7$^{A}$ | 0.52 | 0.49 | 0.15 |
| | 5 | 19.2 ± 2.1$^{B}$ | 15.3 ± 2.0$^{B}$ | 18.7 ± 2.0$^{B}$ | 18.4 ± 1.7$^{B}$ | <0.001 | 0.19 | 0.63 |
| 16:1 *cis*-9 | 1 | 3.06 ± 0.80 | 3.53 ± 0.74 | 2.72 ± 0.78 | 2.82 ± 0.64 | 0.22 | 0.18 | 0.45 |
| | 5 | 5.36 ± 0.95 | 4.35 ± 0.94 | 5.13 ± 0.93 | 2.99 ± 0.79 | <0.01 | 0.04 | 0.75 |

**Supplemental Table S2.6: Continuation**

| Fatty acid, %[3] | Time[4] | Maternal supplementation | | | | *P*-value[2] | | |
|---|---|---|---|---|---|---|---|---|
| | | CTRL | EFA | CLA | EFA+CLA | EFA | CLA | EFA × CLA |
| | | | | | | Time | EFA × time | CLA × time |
| 17:0 | 1 | 1.92 ± 0.22$^{A}$ | 1.85 ± 0.21$^{A}$ | 1.89 ± 0.22$^{A}$ | 1.96 ± 0.18$^{A}$ | 0.53 | 0.37 | 0.75 |
| | 5 | 1.04 ± 0.23$^{B}$ | 0.84 ± 0.22$^{B}$ | 1.22 ± 0.22$^{B}$ | 1.06 ± 0.18$^{B}$ | <0.001 | 0.27 | 0.32 |
| 17:1 *cis*-9 | 1 | 1.15 ± 0.42 | 1.06 ± 0.40 | 0.68 ± 0.41 | 1.46 ± 0.35 | 0.92 | 0.28 | 0.04 |
| | 5 | 1.47 ± 0.36 | 0.59 ± 0.35 | 0.57 ± 0.35 | 0.66 ± 0.29 | 0.23 | 0.10 | 0.39 |
| 18:0 | 1 | 13.10 ± 1.79$^{A}$ | 10.63 ± 1.65$^{A}$ | 13.08 ± 1.73$^{A}$ | 11.49 ± 1.43$^{A}$ | 0.06 | 0.53 | 0.52 |
| | 5 | 8.01 ± 1.79$^{B}$ | 4.82 ± 1.69$^{B}$ | 7.98 ± 1.73$^{B}$ | 6.61 ± 1.43$^{B}$ | <0.001 | 0.83 | 0.70 |
| 18:1 *cis*-9 | 1 | 4.97 ± 1.47 | 6.81 ± 1.39 | 6.33 ± 1.44 | 6.89 ± 1.23 | 0.79 | 0.88 | 0.42 |
| | 5 | 5.32 ± 1.17 | 5.10 ± 1.11 | 5.40 ± 1.13 | 4.03 ± 0.93 | 0.06 | 0.14 | 0.37 |
| 18:1 *cis*-11 | 1 | 1.83 ± 0.50 | 2.15 ± 0.49 | 2.04 ± 0.50 | 1.76 ± 0.44 | 0.84 | 0.79 | 0.48 |
| | 5 | 0.76 ± 0.21 | 0.69 ± 0.20 | 0.77 ± 0.20 | 0.59 ± 0.17 | <0.001 | 0.76 | 0.94 |
| 18:1 *trans*-9 | 1 | 0.26 ± 0.06 | 0.28 ± 0.06$^{A}$ | 0.20 ± 0.06 | 0.25 ± 0.05 | 0.89 | 0.81 | 0.75 |
| | 5 | 0.13 ± 0.05 | 0.10 ± 0.05$^{B}$ | 0.16 ± 0.05 | 0.13 ± 0.04 | <0.001 | 0.18 | 0.11 |
| 18:1 *trans*-11 | 1 | 0.13 ± 0.08 | 0.39 ± 0.08 | 0.38 ± 0.08 | 0.37 ± 0.07 | 0.29 | 0.04 | 0.07 |
| | 5 | 0.16 ± 0.06 | 0.16 ± 0.06 | 0.24 ± 0.06 | 0.19 ± 0.05 | <0.01 | 0.05 | 0.40 |
| 18:2 *cis*-9, *cis*-12 | 1 | 0.00 ± 2.46$^{B*}$ | 3.00 ± 2.29$^{B}$ | 3.46 ± 2.39$^{B}$ | 2.14 ± 2.00$^{B}$ | 0.53 | 0.94 | 0.26 |
| (LA) | 5 | 28.38 ± 4.07$^{A}$ | 31.05 ± 4.16$^{A}$ | 28.02 ± 4.02$^{A}$ | 27.61 ± 3.55$^{A}$ | <0.001 | 0.99 | 0.45 |
| 18:2 *cis*-9, *trans*-11 | 1 | 0.01 ± 0.03 | 0.09 ± 0.03 | 0.07 ± 0.03 | 0.06 ± 0.03 | 0.66 | 0.30 | 0.09 |
| CLA | 5 | 0.06 ± 0.02$^{b}$ | 0.06 ± 0.01$^{ab}$ | 0.10 ± 0.02$^{a}$ | 0.06 ± 0.01$^{b}$ | 0.24 | 0.07 | 0.69 |
| 18:2 *trans*-9, *trans*-12 | 1 | 0.60 ± 0.13 | 0.57 ± 0.13 | 0.49 ± 0.13 | 0.35 ± 0.12 | 0.29 | 0.20 | 0.44 |
| | 5 | 0.26 ± 0.05 | 0.25 ± 0.05 | 0.30 ± 0.05 | 0.20 ± 0.04 | <0.001 | 0.80 | 0.21 |
| 18:3 *cis*-6, *cis*-9, *cis*-12 | 1 | 1.08 ± 0.29 | 0.95 ± 0.27 | 0.90 ± 0.28 | 0.76 ± 0.24 | 0.22 | 0.72 | 0.87 |
| | 5 | 0.59 ± 0.19 | 0.38 ± 0.18 | 0.71 ± 0.18 | 0.42 ± 0.15 | <0.01 | 0.62 | 0.26 |
| 18:3 *cis*-9, *cis*-12, *cis*-15 | 1 | 0.17 ± 0.17 | 0.64 ± 0.16$^{B}$ | 0.77 ± 0.18 | 0.77 ± 0.16$^{B}$ | <0.001 | 0.91 | 0.43 |
| (ALA) | 5 | 2.36 ± 0.87$^{b}$ | 12.00 ± 0.92$^{a,A}$ | 2.54 ± 0.87$^{b}$ | 11.27 ± 0.82$^{a,A}$ | <0.001 | <0.001 | 0.49 |

**Supplemental Table S2.6: Continuation**

| Fatty acid, %[3] | Time[4] | Maternal supplementation | | | | *P*-value[2] | | |
|---|---|---|---|---|---|---|---|---|
| | | CTRL | EFA | CLA | EFA+CLA | EFA<br>Time | CLA<br>EFA × time | EFA × CLA<br>CLA × time |
| 18:4 *cis*-6, *cis*-9, | 1 | 0.03 ± 0.02$^{ab}$ | 0.04 ± 0.01$^{a}$ | 0.01 ± 0.01$^{ab}$ | 0.00 ± 0.01$^{b*}$ | 0.89 | 0.36 | 0.06 |
| *cis*-12, *cis*-15 | 5 | 0.01 ± 0.02 | 0.05 ± 0.02 | 0.06 ± 0.02 | 0.02 ± 0.02 | 0.16 | 0.80 | 0.07 |
| 20:0 | 1 | 4.21 ± 0.58 | 3.70 ± 0.56 | 4.19 ± 0.57 | 3.51 ± 0.49 | 0.11 | 0.90 | 0.88 |
| | 5 | 3.17 ± 0.41 | 2.80 ± 0.39 | 3.20 ± 0.40 | 2.83 ± 0.33 | <0.01 | 0.68 | 0.81 |
| 20:1 *cis*-11 | 1 | 0.27 ± 0.08 | 0.30 ± 0.08$^{A}$ | 0.28 ± 0.08$^{A}$ | 0.29 ± 0.07 | 0.63 | 1.00 | 0.69 |
| | 5 | 0.09 ± 0.06 | 0.07 ± 0.06$^{B}$ | 0.05 ± 0.06$^{B}$ | 0.11 ± 0.05 | <0.001 | 0.95 | 0.92 |
| 20:2 *cis*-11, *cis*-14 | 1 | 0.02 ± 0.05 | 0.08 ± 0.05 | 0.15 ± 0.05 | 0.11 ± 0.04 | 0.51 | <0.05 | 0.73 |
| | 5 | 0.04 ± 0.05 | 0.05 ± 0.05 | 0.05 ± 0.05 | 0.12 ± 0.04 | 0.34 | 0.51 | 0.45 |
| 20:3 *cis*-5, *cis*-8, | 1 | 0.37 ± 0.09 | 0.25 ± 0.09 | 0.21 ± 0.09 | 0.15 ± 0.08 | 0.37 | 0.14 | 0.74 |
| *cis*-11 | 5 | 0.18 ± 0.04 | 0.18 ± 0.04 | 0.16 ± 0.04 | 0.17 ± 0.03 | 0.04 | 0.19 | 0.10 |
| 20:3 *cis*-8, *cis*-11, | 1 | 3.55 ± 0.41 | 4.00 ± 0.40 | 3.11 ± 0.40 | 3.19 ± 0.35 | 0.62 | 0.06 | 0.31 |
| *cis*-14 | 5 | 2.61 ± 0.25 | 2.74 ± 0.23 | 2.71 ± 0.24 | 2.44 ± 0.20 | <0.001 | 0.43 | 0.22 |
| 20:3 *cis*-11, *cis*-14, | 1 | 0.69 ± 0.36 | 0.03 ± 0.36 | 0.01 ± 0.36 | 0.01 ± 0.32 | 0.35 | 0.39 | 0.39 |
| *cis*-17 | 5 | 0.00 ± 0.03$^{*}$ | 0.00 ± 0.03$^{*}$ | 0.03 ± 0.03 | 0.00 ± 0.02$^{*}$ | 0.27 | 0.35 | 0.27 |
| 20:4 *cis*-5, *cis*-8, *cis*-11, | 1 | 3.57 ± 0.92 | 3.35 ± 0.90 | 4.31 ± 0.91 | 3.04 ± 0.81 | 0.15 | 1.00 | 0.58 |
| *cis*-14 (ARA) | 5 | 2.91 ± 0.39$^{a}$ | 2.15 ± 0.36$^{ab}$ | 2.73 ± 0.37$^{ab}$ | 1.90 ± 0.31$^{b}$ | <0.01 | 0.95 | 0.56 |
| 20:5 *cis*-5, *cis*-8, *cis*-11, | 1 | 0.02 ± 0.12$^{b}$ | 0.52 ± 0.12$^{a,B}$ | 0.05 ± 0.13$^{b}$ | 0.49 ± 0.11$^{a}$ | <0.001 | 0.48 | 0.38 |
| *cis*-14, *cis*-17 (EPA) | 5 | 0.26 ± 0.12$^{bc}$ | 0.97 ± 0.12$^{a,A}$ | 0.26 ± 0.12$^{c}$ | 0.68 ± 0.11$^{ab}$ | <0.001 | 0.41 | 0.20 |
| 21:0 | 1 | 1.06 ± 0.14$^{A}$ | 0.90 ± 0.13$^{A}$ | 1.10 ± 0.13$^{A}$ | 0.93 ± 0.11 | 0.13 | 0.20 | 0.89 |
| | 5 | 0.57 ± 0.12$^{B}$ | 0.51 ± 0.11$^{B}$ | 0.75 ± 0.11$^{B}$ | 0.65 ± 0.09 | <0.001 | 0.42 | 0.27 |
| 22:0 | 1 | 3.68 ± 0.54$^{A}$ | 3.06 ± 0.50 | 3.67 ± 0.52 | 3.38 ± 0.44$^{A}$ | 0.29 | 0.32 | 0.96 |
| | 5 | 2.05 ± 0.46$^{B}$ | 2.03 ± 0.43 | 2.70 ± 0.45 | 2.28 ± 0.37$^{B}$ | <0.001 | 0.51 | 0.43 |
| 22:1 *cis*-13 | 1 | 0.03 ± 0.03 | 0.05 ± 0.03 | 0.02 ± 0.03 | 0.00 ± 0.03$^{*}$ | 0.84 | 0.29 | 0.16 |
| | 5 | 0.01 ± 0.02 | 0.02 ± 0.02 | 0.02 ± 0.02 | 0.00 ± 0.02$^{*}$ | 0.29 | 0.69 | 0.52 |

**Supplemental Table S2.6: Continuation**

| Fatty acid, %[3] | Time[4] | Maternal supplementation | | | | *P*-value[2] | | |
|---|---|---|---|---|---|---|---|---|
| | | CTRL | EFA | CLA | EFA+CLA | EFA<br>Time | CLA<br>EFA × time | EFA × CLA<br>CLA × time |
| 22:2 *cis*-13, *cis*-16 | 1 | 0.63 ± 0.27 | 0.71 ± 0.24 | 0.81 ± 0.26 | 0.92 ± 0.21 | 0.69 | 0.15 | 0.98 |
| | 5 | 0.43 ± 0.25 | 0.49 ± 0.24 | 0.73 ± 0.25 | 0.74 ± 0.20 | <0.01 | 0.62 | 0.53 |
| 22:4 *cis*-7, *cis*-10, *cis*- | 1 | 0.03 ± 0.04 | 0.02 ± 0.04 | 0.04 ± 0.04 | 0.00 ± 0.04* | 0.76 | 0.96 | 0.53 |
| 13, *cis*-16 | 5 | 0.00 ± 0.04* | 0.02 ± 0.04 | 0.02 ± 0.04 | 0.01 ± 0.03 | 0.13 | 0.14 | 0.47 |
| 22:5 *cis*-4, *cis*-7, *cis*-10, | 1 | 0.09 ± 0.04 | 0.01 ± 0.04 | 0.03 ± 0.04 | 0.00 ± 0.03* | 0.11 | 0.39 | 0.54 |
| *cis*-13, *cis*-16 | 5 | 0.02 ± 0.03 | 0.00 ± 0.02* | 0.03 ± 0.02 | 0.00 ± 0.02* | 0.24 | 0.29 | 0.19 |
| 22:5 *cis*-7, *cis*-10, *cis*-13, | 1 | 4.00 ± 0.77[A] | 3.36 ± 0.72 | 4.05 ± 0.75 | 3.91 ± 0.63 | 0.47 | 0.31 | 0.82 |
| *cis*-16, *cis*-19 (DPA) | 5 | 2.42 ± 0.66[B] | 2.17 ± 0.61 | 3.07 ± 0.63 | 2.74 ± 0.52 | <0.001 | 0.84 | 0.52 |
| 22:6 *cis*-4, *cis*-7, *cis*-10, | 1 | 0.76 ± 0.35 | 0.71 ± 0.34 | 1.01 ± 0.35 | 0.32 ± 0.30 | 0.21 | 1.00 | 0.26 |
| *cis*-13, *cis*-16, *cis*-19 (DHA) | 5 | 0.26 ± 0.26 | 0.25 ± 0.24 | 0.47 ± 0.25 | 0.18 ± 0.20 | <0.01 | 0.37 | 0.56 |
| 23:0 | 1 | 2.63 ± 0.71 | 1.36 ± 0.66 | 1.64 ± 0.69 | 1.42 ± 0.58 | 0.92 | 0.14 | 0.65 |
| | 5 | 0.92 ± 0.69 | 1.91 ± 0.66 | 0.37 ± 0.66 | 1.03 ± 0.55 | 0.03 | 0.01 | 0.68 |
| 24:0 | 1 | 5.17 ± 0.90[A] | 4.37 ± 0.85 | 5.35 ± 0.88 | 5.07 ± 0.75 | 0.54 | 0.27 | 0.99 |
| | 5 | 3.06 ± 0.74[B] | 3.17 ± 0.69 | 4.02 ± 0.71 | 3.65 ± 0.58 | <0.001 | 0.44 | 0.61 |
| SFA[5] | 1 | 77.7 ± 4.9[A] | 69.6 ± 4.5[A] | 72.5 ± 4.7[A] | 72.9 ± 3.9[A] | 0.08 | 0.60 | 0.17 |
| | 5 | 50.3 ± 5.8[B] | 38.5 ± 5.7[B] | 50.2 ± 5.6[B] | 46.7 ± 4.8[B] | <0.001 | 0.46 | 0.34 |
| MUFA[6] | 1 | 12.68 ± 2.80 | 14.84 ± 2.66 | 13.23 ± 2.74 | 14.49 ± 2.35 | 0.59 | 0.61 | 0.79 |
| | 5 | 14.22 ± 2.08 | 11.17 ± 1.96 | 12.95 ± 2.00 | 9.29 ± 1.64 | 0.11 | 0.04 | 0.48 |
| PUFA[7] | 1 | 11.7 ± 2.8[B] | 16.1 ± 2.6[B] | 16.3 ± 2.7[B] | 13.9 ± 2.2[B] | 0.02 | 0.81 | 0.11 |
| | 5 | 37.6 ± 4.5[A] | 50.9 ± 4.6[A] | 38.8 ± 4.4[A] | 45.4 ± 3.9[A] | <0.001 | 0.07 | 0.49 |
| Sum of n-3 fatty acids[8] | 1 | 4.04 ± 0.95 | 3.54 ± 0.88[B] | 3.64 ± 0.92 | 4.26 ± 0.77[B] | <0.001 | 0.98 | 0.91 |
| | 5 | 3.67 ± 1.01[b] | 13.62 ± 0.99[a,A] | 4.17 ± 0.99[b] | 12.75 ± 0.83[a,A] | <0.001 | <0.001 | 0.73 |
| Sum of n-6 fatty acids[9] | 1 | 6.32 ± 2.76[B] | 11.56 ± 2.55[B] | 11.55 ± 2.67[B] | 8.95 ± 2.21[B] | 0.59 | 0.88 | 0.10 |
| | 5 | 33.02 ± 4.14[A] | 36.53 ± 4.21[A] | 33.73 ± 4.08[A] | 32.02 ± 3.56[A] | <0.001 | 0.92 | 0.46 |

**Supplemental Table S2.6: Continuation**

| Fatty acid, %[3] | Time[4] | Maternal supplementation | | | | *P*-value[2] | | |
|---|---|---|---|---|---|---|---|---|
| | | CTRL | EFA | CLA | EFA+CLA | EFA | CLA | EFA × CLA |
| | | | | | | Time | EFA × time | CLA × time |
| Ratio n-6:n-3 fatty acids | 1 | 5.72 ± 1.56[B] | 4.12 ± 1.46 | 6.84 ± 1.52 | 3.20 ± 1.28 | <0.001 | 0.63 | 0.72 |
| | 5 | 10.46 ± 1.38[a,A] | 3.36 ± 1.30[b] | 9.13 ± 1.33[a] | 2.83 ± 1.09[b] | 0.02 | <0.01 | 0.39 |

[a-c] LSM within a row with different lowercase letters differ between treatments ($P < 0.05$).

[A, B] LSM within a column with different uppercase letters differ between days of life ($P < 0.05$).

[*] LSM were below the detection limit of 0.01%.

[1] Values are presented as LSM ± SE.

[2] *P*-values for fixed effects are presented in 2 rows: The first row indicates *P*-values for the effect of EFA, CLA, and their interaction; the second row indicates *P*-values for the effect of time and interactions between EFA or CLA and time.

[3] Proportion of the presented fatty acid in total cholesterol esters.

[4] Day of life.

[5] Sum of saturated fatty acids, consisting of 10:0; 11:0; 12:0; 13:0; 14:0; 15:0; 16:0; 17:0; 18:0; 20:0; 21:0; 22:0; 23:0; 24:0.

[6] Sum of monounsaturated fatty acids, consisting of 14:1 *cis*-9; 16:1 *cis*-9; 17:1 *cis*-9; 18:1 *cis*-9; 18:1 *cis*-11; 18:1 *trans*-9; 18:1 *trans*-11; 20:1 *cis*-11; 22:1 *cis*-13.

[7] Sum of polyunsaturated fatty acids, consisting of 18:2 *cis*-9, *cis*-12; 18:2 *cis*-9, *trans*-11; 18:2 *trans*-9, *trans*-12; 18:3 *cis*-6, *cis*-9, *cis*-12; 18:3 *cis*-9, *cis*-12, *cis*-15; 18:4 *cis*-6, *cis*-9, *cis*-12, *cis*-15; 20:2 *cis*-11, *cis*-14; 20:3 *cis*-5, *cis*-8, *cis*-11; 20:3 *cis*-8, *cis*-11, *cis*-14; 20:3 *cis*-11, *cis*-14, *cis*-17; 20:4 *cis*-5, *cis*-8, *cis*-11, *cis*-14; 20:5 *cis*-5, *cis*-8, *cis*-11, *cis*-14, *cis*-17; 22:2 *cis*-13, *cis*-16; 22:4 *cis*-7, *cis*-10, *cis*-13, *cis*-16; 22:5 *cis*-4, *cis*-7, *cis*-10, *cis*-13, *cis*-16; 22:5 *cis*-7, *cis*-10, *cis*-13, *cis*-16, *cis*-19; 22:6 *cis*-4, *cis*-7, *cis*-10, *cis*-13, *cis*-16, *cis*-19.

[8] Sum of n-3 fatty acids, consisting of 18:3 *cis*-9, *cis*-12, *cis*-15; 18:4 *cis*-6, *cis*-9, *cis*-12, *cis*-15; 20:3 *cis*-11, *cis*-14, *cis*-17; 20:5 *cis*-5, *cis*-8, *cis*-11, *cis*-14, *cis*-17; 22:5 *cis*-7, *cis*-10, *cis*-13, *cis*-16, *cis*-19; 22:6 *cis*-4, *cis*-7, *cis*-10, *cis*-13, *cis*-16, *cis*-19.

[9] Sum of n-6 fatty acids, consisting of 18:2 *cis*-9, *cis*-12; 18:3 *cis*-6, *cis*-9, *cis*-12; 20:2 *cis*-11, *cis*-14; 20:3 *cis*-8, *cis*-11, *cis*-14; 20:4 *cis*-5, *cis*-8, *cis*-11, *cis*-14; 22:2 *cis*-13, *cis*-16; 22:4 *cis*-7, *cis*-10, *cis*-13, *cis*-16; 22:5 *cis*-4, *cis*-7, *cis*-10, *cis*-13, *cis*-16.

**Supplemental Table S2.7: Effects of the maternal supplementation with coconut oil (CTRL; n = 9), linseed and safflower oil (EFA; n = 9), Lutalin (CLA; *cis*-9, *trans*-11 and *trans*-10, *cis*-12; n = 9), or a combination of the EFA and CLA supplement (EFA+CLA; n = 11) on the fatty acid composition in plasma free fatty acids of calves on d 1 and 5 of life[1]**

| | | Maternal supplementation | | | | *P*-value[2] | | |
|---|---|---|---|---|---|---|---|---|
| Fatty acid, %[3] | Time[4] | CTRL | EFA | CLA | EFA+CLA | EFA<br>Time | CLA<br>EFA × time | EFA × CLA<br>CLA × time |
| 10:0 | 1 | 0.37 ± 0.29 | 0.35 ± 0.28 | 0.86 ± 0.29 | 0.34 ± 0.25 | 0.35 | 0.58 | 0.32 |
| | 5 | 0.51 ± 0.19 | 0.55 ± 0.17 | 0.52 ± 0.18 | 0.42 ± 0.15 | 0.85 | 0.31 | 0.19 |
| 11:0 | 1 | 0.08 ± 0.05 | 0.04 ± 0.04 | 0.02 ± 0.05 | 0.05 ± 0.04 | 0.26 | 0.90 | 0.77 |
| | 5 | 0.14 ± 0.08 | 0.04 ± 0.08 | 0.16 ± 0.08 | 0.05 ± 0.07 | 0.07 | 0.06 | 0.57 |
| 12:0 | 1 | 1.14 ± 0.41 | 0.57 ± 0.38 | 0.48 ± 0.40 | 0.21 ± 0.33 | 0.72 | 0.19 | 0.94 |
| | 5 | 0.78 ± 0.45 | 1.12 ± 0.43 | 0.72 ± 0.44 | 0.83 ± 0.36 | 0.06 | 0.03 | 0.23 |
| 13:0 | 1 | 0.09 ± 0.02 | 0.08 ± 0.02 | 0.11 ± 0.02 | 0.09 ± 0.01 | 0.32 | 0.14 | 0.52 |
| | 5 | 0.11 ± 0.01 | 0.11 ± 0.01 | 0.13 ± 0.01 | 0.12 ± 0.01 | <0.001 | 0.25 | 0.98 |
| 14:0 | 1 | 2.38 ± 0.27$^{B}$ | 1.96 ± 0.25$^{B}$ | 2.45 ± 0.26$^{B}$ | 2.16 ± 0.22$^{B}$ | 0.12 | 0.59 | 0.90 |
| | 5 | 3.59 ± 0.32$^{A}$ | 3.43 ± 0.32$^{A}$ | 3.65 ± 0.31$^{A}$ | 3.44 ± 0.27$^{A}$ | <0.001 | 0.61 | 0.77 |
| 14:1 *cis*-9 | 1 | 0.25 ± 0.06 | 0.20 ± 0.06 | 0.21 ± 0.06 | 0.13 ± 0.05 | 0.26 | 0.09 | 0.44 |
| | 5 | 0.14 ± 0.05 | 0.16 ± 0.05 | 0.11 ± 0.05 | 0.08 ± 0.04 | 0.02 | 0.35 | 0.95 |
| 15:0 | 1 | 0.63 ± 0.13 | 0.50 ± 0.12 | 0.72 ± 0.13 | 0.59 ± 0.11 | 0.39 | 0.20 | 0.83 |
| | 5 | 0.77 ± 0.11 | 0.80 ± 0.10 | 0.88 ± 0.11 | 0.86 ± 0.09 | <0.001 | 0.19 | 0.93 |
| 16:0 | 1 | 43.6 ± 1.9 | 39.8 ± 1.8 | 42.7 ± 1.9 | 43.6 ± 1.5 | 0.51 | 0.24 | 0.40 |
| | 5 | 44.1 ± 1.8 | 44.6 ± 1.7 | 45.8 ± 1.7 | 45.2 ± 1.4 | <0.01 | 0.32 | 0.82 |
| 16:1 *cis*-9 | 1 | 3.24 ± 0.69 | 3.92 ± 0.68$^{A}$ | 3.18 ± 0.68$^{A}$ | 3.03 ± 0.61$^{A}$ | 0.86 | 0.42 | 0.77 |
| | 5 | 1.40 ± 0.24 | 1.06 ± 0.22$^{B}$ | 1.06 ± 0.23$^{B}$ | 1.13 ± 0.19$^{B}$ | <0.001 | 0.49 | 0.56 |

**Supplemental Table S2.7: Continuation**

| Fatty acid, %[3] | Time[4] | Maternal supplementation | | | | *P*-value[2] | | |
|---|---|---|---|---|---|---|---|---|
| | | CTRL | EFA | CLA | EFA+CLA | EFA | CLA | EFA × CLA |
| | | | | | | Time | EFA × time | CLA × time |
| 17:0 | 1 | 1.21 ± 0.12 | 1.25 ± 0.11 | 1.32 ± 0.11 | 1.30 ± 0.09 | 0.73 | 0.30 | 0.80 |
| | 5 | 1.43 ± 0.12 | 1.48 ± 0.12 | 1.50 ± 0.12 | 1.54 ± 0.10 | <0.001 | 0.66 | 0.89 |
| 17:1 *cis*-9 | 1 | 0.23 ± 0.05 | 0.35 ± 0.05$^{A}$ | 0.25 ± 0.05 | 0.25 ± 0.05 | 0.89 | 0.13 | 0.87 |
| | 5 | 0.22 ± 0.04 | 0.13 ± 0.04$^{B}$ | 0.13 ± 0.04 | 0.13 ± 0.03 | <0.001 | 0.02 | 0.95 |
| 18:0 | 1 | 21.3 ± 1.9 | 19.2 ± 1.8 | 21.0 ± 1.9 | 20.8 ± 1.6 | 0.73 | 0.42 | 0.69 |
| | 5 | 23.6 ± 1.6 | 24.2 ± 1.5 | 24.8 ± 1.5 | 25.0 ± 1.2 | <0.001 | 0.31 | 0.81 |
| 18:1 *cis*-9 | 1 | 14.33 ± 3.10 | 19.95 ± 2.97$^{A}$ | 14.27 ± 3.04 | 16.12 ± 2.63$^{A}$ | 0.44 | 0.21 | 0.92 |
| | 5 | 10.38 ± 2.16 | 6.99 ± 2.03$^{B}$ | 6.09 ± 2.08 | 7.10 ± 1.70$^{B}$ | <0.001 | 0.07 | 0.96 |
| 18:1 *cis*-11 | 1 | 1.42 ± 0.30 | 2.03 ± 0.29$^{A}$ | 1.61 ± 0.29$^{A}$ | 1.61 ± 0.26$^{A}$ | 0.52 | 0.43 | 0.64 |
| | 5 | 0.81 ± 0.18 | 0.55 ± 0.16$^{B}$ | 0.52 ± 0.17$^{B}$ | 0.58 ± 0.14$^{B}$ | <0.001 | 0.12 | 0.93 |
| 18:1 *trans*-9 | 1 | 0.20 ± 0.06 | 0.22 ± 0.06 | 0.12 ± 0.06 | 0.25 ± 0.05 | 0.28 | 0.65 | 0.01 |
| | 5 | 0.18 ± 0.05 | 0.07 ± 0.05 | 0.07 ± 0.05 | 0.19 ± 0.04 | <0.01 | 0.13 | 0.52 |
| 18:1 *trans*-11 | 1 | 0.06 ± 0.01$^{B}$ | 0.04 ± 0.01 | 0.03 ± 0.01$^{B}$ | 0.05 ± 0.01$^{B}$ | 0.25 | 0.69 | <0.01 |
| | 5 | 0.20 ± 0.03$^{A}$ | 0.09 ± 0.04 | 0.15 ± 0.03$^{A}$ | 0.19 ± 0.03$^{A}$ | <0.001 | 0.39 | 0.24 |
| 18:2 *cis*-9, *cis*-12 | 1 | 0.63 ± 0.21$^{B}$ | 1.00 ± 0.19 | 0.63 ± 0.20 | 0.73 ± 0.16 | 0.56 | 0.23 | 0.79 |
| LA | 5 | 1.57 ± 0.26$^{A}$ | 1.29 ± 0.26 | 1.16 ± 0.26 | 1.31 ± 0.22 | <0.001 | 0.19 | 0.78 |
| 18:2 *cis*-9, *trans*-11 | 1 | 0.04 ± 0.01$^{a}$ | 0.01 ± 0.01$^{b}$ | 0.03 ± 0.01$^{ab}$ | 0.02 ± 0.01$^{ab}$ | 0.02 | 0.59 | 0.59 |
| CLA | 5 | 0.06 ± 0.01 | 0.04 ± 0.01 | 0.07 ± 0.01 | 0.05 ± 0.01 | <0.001 | 0.93 | 0.20 |
| 18:2 *trans*-9, *trans*-12 | 1 | 0.17 ± 0.06 | 0.12 ± 0.06$^{B}$ | 0.21 ± 0.06 | 0.07 ± 0.05 | 0.51 | 0.42 | <0.05 |
| | 5 | 0.16 ± 0.07 | 0.31 ± 0.07$^{A}$ | 0.21 ± 0.07 | 0.13 ± 0.06 | 0.03 | 0.02 | 0.26 |
| 18:3 *cis*-6, *cis*-9, *cis*-12 | 1 | 0.00 ± 0.03$^{b*}$ | 0.07 ± 0.02$^{a,B}$ | 0.01 ± 0.02$^{ab}$ | 0.04 ± 0.02$^{ab,B}$ | <0.001 | 0.99 | 0.74 |
| | 5 | 0.10 ± 0.09$^{b}$ | 0.50 ± 0.09$^{a,A}$ | 0.07 ± 0.09$^{b}$ | 0.56 ± 0.08$^{a,A}$ | <0.001 | <0.001 | 0.77 |
| 18:3 *cis*-9, *cis*-12, *cis*-15 | 1 | 0.09 ± 0.01 | 0.11 ± 0.01 | 0.09 ± 0.01 | 0.09 ± 0.01 | 0.50 | 0.83 | 0.26 |
| ALA | 5 | 0.07 ± 0.01 | 0.10 ± 0.01 | 0.10 ± 0.01 | 0.09 ± 0.01 | 0.46 | 0.80 | 0.17 |

**Supplemental Table S2.7: Continuation**

| Fatty acid, %[3] | Time[4] | Maternal supplementation | | | | *P*-value[2] | | |
|---|---|---|---|---|---|---|---|---|
| | | CTRL | EFA | CLA | EFA+CLA | EFA | CLA | EFA × CLA |
| | | | | | | Time | EFA × time | CLA × time |
| 18:4 *cis*-6, *cis*-9, | 1 | 0.00 ± 0.00* | 0.01 ± 0.00 | 0.00 ± 0.00* | 0.00 ± 0.00* | 0.48 | 0.94 | 0.70 |
| *cis*-12, *cis*-15 | 5 | 0.00 ± 0.01* | 0.01 ± 0.01 | 0.01 ± 0.01 | 0.01 ± 0.01 | 0.24 | 0.80 | 0.19 |
| 20:0 | 1 | 0.82 ± 0.14 | 0.75 ± 0.13[B] | 1.07 ± 0.14 | 0.79 ± 0.12 | 0.29 | 0.29 | 0.06 |
| | 5 | 1.00 ± 0.11 | 1.20 ± 0.10[A] | 1.19 ± 0.10 | 1.03 ± 0.08 | <0.001 | 0.11 | 0.27 |
| 20:1 *cis*-11 | 1 | 0.03 ± 0.01 | 0.06 ± 0.01 | 0.05 ± 0.01 | 0.04 ± 0.01 | 0.65 | 0.35 | 0.44 |
| | 5 | 0.04 ± 0.01 | 0.03 ± 0.01 | 0.03 ± 0.01 | 0.03 ± 0.01 | 0.02 | 0.18 | 0.57 |
| 20:2 *cis*-11, *cis*-14 | 1 | 0.06 ± 0.02 | 0.04 ± 0.02 | 0.03 ± 0.02 | 0.04 ± 0.02 | 0.79 | 0.98 | 0.45 |
| | 5 | 0.02 ± 0.02 | 0.01 ± 0.02 | 0.03 ± 0.02 | 0.03 ± 0.01 | <0.01 | 0.82 | 0.05 |
| 20:3 *cis*-5, *cis*-8, | 1 | 0.01 ± 0.01 | 0.01 ± 0.01 | 0.01 ± 0.01 | 0.00 ± 0.01* | 0.72 | 0.50 | 0.18 |
| *cis*-11 | 5 | 0.00 ± 0.01* | 0.01 ± 0.01 | 0.01 ± 0.01 | 0.00 ± 0.01* | 0.13 | 0.18 | 0.61 |
| 20:3 *cis*-8, *cis*-11, | 1 | 0.36 ± 0.11 | 0.34 ± 0.10 | 0.30 ± 0.11 | 0.35 ± 0.09 | 0.67 | 0.56 | 0.50 |
| *cis*-14 | 5 | 0.29 ± 0.10 | 0.44 ± 0.09 | 0.35 ± 0.10 | 0.28 ± 0.08 | 0.91 | 0.79 | 0.69 |
| 20:3 *cis*-11, *cis*-14, | 1 | 0.02 ± 0.01 | 0.00 ± 0.01* | 0.02 ± 0.01 | 0.02 ± 0.01 | 0.75 | 0.10 | 0.67 |
| *cis*-17 | 5 | 0.00 ± 0.01* | 0.01 ± 0.01 | 0.02 ± 0.01 | 0.03 ± 0.01 | 0.90 | 0.20 | 0.98 |
| 20:4 *cis*-5, *cis*-8, *cis*-11, | 1 | 0.23 ± 0.12 | 0.15 ± 0.11 | 0.29 ± 0.12 | 0.18 ± 0.09 | 0.36 | 0.99 | 0.28 |
| *cis*-14 (ARA) | 5 | 0.18 ± 0.13 | 0.28 ± 0.12 | 0.28 ± 0.12 | 0.09 ± 0.10 | 0.87 | 0.52 | 0.20 |
| 20:5 *cis*-5, *cis*-8, *cis*-11, | 1 | 0.00 ± 0.00* | 0.01 ± 0.00 | 0.00 ± 0.00* | 0.00 ± 0.00* | 0.17 | 0.48 | 0.38 |
| *cis*-14, *cis*-17 (EPA) | 5 | 0.00 ± 0.01* | 0.02 ± 0.01 | 0.00 ± 0.01* | 0.00 ± 0.01* | 0.26 | 0.26 | 0.30 |
| 21:0 | 1 | 0.50 ± 0.08 | 0.48 ± 0.07[B] | 0.56 ± 0.08 | 0.51 ± 0.06 | 0.92 | 0.30 | 0.19 |
| | 5 | 0.59 ± 0.08 | 0.75 ± 0.07[A] | 0.74 ± 0.07 | 0.68 ± 0.06 | <0.001 | 0.18 | 0.90 |
| 22:0 | 1 | 1.76 ± 0.27 | 1.72 ± 0.25[B] | 2.06 ± 0.26 | 1.90 ± 0.22 | 0.78 | 0.19 | 0.27 |
| | 5 | 2.08 ± 0.29 | 2.57 ± 0.28[A] | 2.56 ± 0.28 | 2.45 ± 0.24 | <0.001 | 0.23 | 0.80 |
| 22:2 *cis*-13, *cis*-16 | 1 | 0.24 ± 0.12 | 0.12 ± 0.11 | 0.21 ± 0.11 | 0.31 ± 0.09 | 0.67 | 0.11 | 0.24 |
| | 5 | 0.21 ± 0.16 | 0.18 ± 0.16 | 0.33 ± 0.16 | 0.56 ± 0.14 | 0.02 | 0.23 | 0.06 |

**Supplemental Table S2.7: Continuation**

| Fatty acid, %[3] | Time[4] | Maternal supplementation | | | | *P*-value[2] | | |
|---|---|---|---|---|---|---|---|---|
| | | CTRL | EFA | CLA | EFA+CLA | EFA<br>Time | CLA<br>EFA × time | EFA × CLA<br>CLA × time |
| 22:4 *cis*-7, *cis*-10, *cis*-13, *cis*-16 | 1 | 0.09 ± 0.05 | 0.04 ± 0.05 | 0.05 ± 0.05 | 0.01 ± 0.04 | 0.15 | 0.56 | 0.79 |
| | 5 | 0.07 ± 0.05 | 0.00 ± 0.05* | 0.05 ± 0.05 | 0.01 ± 0.04 | 0.11 | 0.56 | 0.03 |
| 22:5 *cis*-4, *cis*-7, *cis*-10, *cis*-13, *cis*-16 | 1 | 0.08 ± 0.04 | 0.00 ± 0.04* | 0.01 ± 0.04 | 0.00 ± 0.03* | 0.21 | 0.55 | 0.32 |
| | 5 | 0.01 ± 0.02 | 0.00 ± 0.02* | 0.01 ± 0.02 | 0.01 ± 0.01 | 0.38 | 0.28 | 0.31 |
| 22:5 *cis*-7, *cis*-10, *cis*-13, *cis*-16, *cis*-19 (DPA) | 1 | 1.73 ± 0.30 | 1.75 ± 0.28 | 2.03 ± 0.29 | 2.04 ± 0.24 | 0.58 | 0.10 | 0.52 |
| | 5 | 2.00 ± 0.33 | 2.44 ± 0.32 | 2.58 ± 0.32 | 2.55 ± 0.27 | <0.001 | 0.39 | 0.83 |
| 22:6 *cis*-4, *cis*-7, *cis*-10, *cis*-13, *cis*-16, *cis*-19 (DHA) | 1 | 0.09 ± 0.02 | 0.10 ± 0.02 | 0.10 ± 0.02 | 0.08 ± 0.02 | 0.39 | 0.86 | 0.13 |
| | 5 | 0.09 ± 0.02 | 0.14 ± 0.02 | 0.11 ± 0.02 | 0.11 ± 0.02 | 0.02 | 0.08 | 0.92 |
| 23:0 | 1 | 0.69 ± 0.11 | 0.68 ± 0.10[B] | 0.79 ± 0.11 | 0.78 ± 0.09 | 0.69 | 0.15 | 0.47 |
| | 5 | 0.82 ± 0.12 | 0.99 ± 0.12[A] | 1.02 ± 0.12 | 0.99 ± 0.10 | <0.001 | 0.36 | 0.98 |
| 24:0 | 1 | 2.14 ± 0.38 | 2.15 ± 0.35 | 2.54 ± 0.37 | 2.51 ± 0.30 | 0.61 | 0.11 | 0.54 |
| | 5 | 2.52 ± 0.42 | 3.06 ± 0.41 | 3.19 ± 0.41 | 3.19 ± 0.34 | <0.001 | 0.32 | 0.93 |
| SFA[5] | 1 | 76.4 ± 4.0 | 69.1 ± 3.9[B] | 76.3 ± 4.0 | 74.6 ± 3.4[B] | 0.38 | 0.22 | 0.90 |
| | 5 | 81.8 ± 2.8 | 84.7 ± 2.7[A] | 86.5 ± 2.7 | 84.9 ± 2.2[A] | <0.001 | 0.15 | 0.94 |
| MUFA[6] | 1 | 19.74 ± 4.15 | 26.73 ± 4.00[A] | 19.64 ± 4.08[A] | 21.47 ± 3.55[A] | 0.51 | 0.24 | 0.95 |
| | 5 | 13.35 ± 2.68 | 9.02 ± 2.50[B] | 8.08 ± 2.57[B] | 9.42 ± 2.10[B] | <0.001 | 0.10 | 0.95 |
| PUFA[7] | 1 | 3.91 ± 0.60[B] | 4.00 ± 0.55[B] | 4.11 ± 0.58[B] | 4.10 ± 0.47[B] | 0.39 | 0.55 | 0.73 |
| | 5 | 4.92 ± 0.65[A] | 5.82 ± 0.61[A] | 5.46 ± 0.63[A] | 5.92 ± 0.52[A] | <0.001 | 0.02 | 0.53 |
| Sum of n-3 fatty acids[8] | 1 | 1.79 ± 0.31 | 1.90 ± 0.29[B] | 2.09 ± 0.30 | 2.14 ± 0.25[B] | 0.05 | 0.12 | 0.49 |
| | 5 | 2.15 ± 0.32[b] | 3.09 ± 0.31[a,A] | 2.71 ± 0.31[ab] | 3.20 ± 0.26[a,A] | <0.001 | <0.01 | 0.74 |
| Sum of n-6 fatty acids[9] | 1 | 1.88 ± 0.34[B] | 1.95 ± 0.31 | 1.73 ± 0.33[B] | 1.80 ± 0.27[B] | 0.99 | 0.72 | 0.84 |
| | 5 | 2.54 ± 0.38[A] | 2.39 ± 0.36 | 2.44 ± 0.37[A] | 2.46 ± 0.31[A] | <0.001 | 0.44 | 0.46 |

**Supplemental Table S2.7: Continuation**

| Fatty acid, %[3] | Time[4] | Maternal supplementation | | | | *P*-value[2] | | |
|---|---|---|---|---|---|---|---|---|
| | | CTRL | EFA | CLA | EFA+CLA | EFA | CLA | EFA × CLA |
| | | | | | | Time | EFA × time | CLA × time |
| Ratio n-6:n-3 fatty acids | 1 | 1.21 ± 0.19 | 1.20 ± 0.18 | 1.07 ± 0.19 | 0.99 ± 0.16 | 0.19 | 0.10 | 0.73 |
| | 5 | 1.27 ± 0.17[a] | 0.90 ± 0.16[ab] | 0.97 ± 0.17[ab] | 0.83 ± 0.14[b] | 0.11 | 0.17 | 0.94 |

[a-c] LSM within a row with different lowercase letters differ between treatments ($P < 0.05$).

[A, B] LSM within a column with different uppercase letters differ between days of life ($P < 0.05$).

[*] LSM were below the detection limit of 0.01%.

[1] Values are presented as LSM ± SE.

[2] *P*-values for fixed effects are presented in 2 rows: The first row indicates *P*-values for the effect of EFA, CLA, and their interaction; the second row indicates *P*-values for the effect of time and interactions between EFA or CLA and time.

[3] Proportion of the presented fatty acid in total free fatty acids.

[4] Day of life.

[5] Sum of saturated fatty acids, consisting of 10:0; 11:0; 12:0; 13:0; 14:0; 15:0; 16:0; 17:0; 18:0; 20:0; 21:0; 22:0; 23:0; 24:0.

[6] Sum of monounsaturated fatty acids, consisting of 14:1 *cis*-9; 16:1 *cis*-9; 17:1 *cis*-9; 18:1 *cis*-9; 18:1 *cis*-11; 18:1 *trans*-9; 18:1 *trans*-11; 20:1 *cis*-11; 22:1 *cis*-13.

[7] Sum of polyunsaturated fatty acids, consisting of 18:2 *cis*-9, *cis*-12; 18:2 *cis*-9, *trans*-11; 18:2 *trans*-9, *trans*-12; 18:3 *cis*-6, *cis*-9, *cis*-12; 18:3 *cis*-9, *cis*-12, *cis*-15; 18:4 *cis*-6, *cis*-9, *cis*-12, *cis*-15; 20:2 *cis*-11, *cis*-14; 20:3 *cis*-5, *cis*-8, *cis*-11; 20:3 *cis*-8, *cis*-11, *cis*-14; 20:3 *cis*-11, *cis*-14, *cis*-17; 20:4 *cis*-5, *cis*-8, *cis*-11, *cis*-14; 20:5 *cis*-5, *cis*-8, *cis*-11, *cis*-14, *cis*-17; 22:2 *cis*-13, *cis*-16; 22:4 *cis*-7, *cis*-10, *cis*-13, *cis*-16; 22:5 *cis*-4, *cis*-7, *cis*-10, *cis*-13, *cis*-16; 22:5 *cis*-7, *cis*-10, *cis*-13, *cis*-16, *cis*-19; 22:6 *cis*-4, *cis*-7, *cis*-10, *cis*-13, *cis*-16, *cis*-19.

[8] Sum of n-3 fatty acids, consisting of 18:3 *cis*-9, *cis*-12, *cis*-15; 18:4 *cis*-6, *cis*-9, *cis*-12, *cis*-15; 20:3 *cis*-11, *cis*-14, *cis*-17; 20:5 *cis*-5, *cis*-8, *cis*-11, *cis*-14, *cis*-17; 22:5 *cis*-7, *cis*-10, *cis*-13, *cis*-16, *cis*-19; 22:6 *cis*-4, *cis*-7, *cis*-10, *cis*-13, *cis*-16, *cis*-19.

[9] Sum of n-6 fatty acids, consisting of 18:2 *cis*-9, *cis*-12; 18:3 *cis*-6, *cis*-9, *cis*-12; 20:2 *cis*-11, *cis*-14; 20:3 *cis*-8, *cis*-11, *cis*-14; 20:4 *cis*-5, *cis*-8, *cis*-11, *cis*-14; 22:2 *cis*-13, *cis*-16; 22:4 *cis*-7, *cis*-10, *cis*-13, *cis*-16; 22:5 *cis*-4, *cis*-7, *cis*-10, *cis*-13, *cis*-16.

**Supplemental Table S3.1: Organ weights in calves, whose dams were supplemented with coconut oil (CTRL; n = 9), linseed and safflower oil (EFA; n = 9), Lutalin (CLA; *cis*-9, *trans*-11 and *trans*-10, *cis*-12; n = 9), or a combination of the EFA and CLA supplement (EFA+CLA; n = 11)[1]**

| | Maternal supplementation | | | | *P*-value | | |
|---|---|---|---|---|---|---|---|
| Item[2] | CTRL | EFA | CLA | EFA+CLA | EFA | CLA | EFA × CLA |
| Liver, g | 1139 ± 70 | 1141 ± 62 | 1074 ± 65 | 1178 ± 54 | 0.25 | 0.74 | 0.25 |
| Liver, g/kg BW | 25.5 ± 1.6 | 25.6 ± 1.5 | 24.2 ± 1.5 | 26.5 ± 1.3 | 0.27 | 0.83 | 0.28 |
| Kidney, g | 104.7 ± 6.3 | 102.6 ± 5.7 | 98.7 ± 5.9 | 103.8 ± 5.0 | 0.71 | 0.54 | 0.37 |
| Kidney, g/kg BW | 2.35 ± 0.14 | 2.32 ± 0.13 | 2.23 ± 0.13 | 2.33 ± 0.11 | 0.72 | 0.55 | 0.43 |
| Pancreas, g | 49.9 ± 5.1 | 49.6 ± 4.6 | 45.9 ± 4.8 | 44.6 ± 4.0 | 0.80 | 0.16 | 0.87 |
| Pancreas, g/kg BW | 1.10 ± 0.11 | 1.08 ± 0.10 | 1.02 ± 0.11 | 0.98 ± 0.09 | 0.72 | 0.25 | 0.87 |
| Spleen, g | 110 ± 11 | 107 ± 10 | 112 ± 11 | 110 ± 9 | 0.76 | 0.73 | 0.96 |
| Spleen, g/kg BW | 2.60 ± 0.25 | 2.49 ± 0.23 | 2.55 ± 0.24 | 2.55 ± 0.20 | 0.72 | 0.97 | 0.73 |
| Thymus, g | 103.8 ± 17.7 | 99.5 ± 15.8 | 107.2 ± 16.6 | 110.7 ± 13.8 | 0.97 | 0.51 | 0.72 |
| Thymus, g/kg BW | 2.46 ± 0.39 | 2.35 ± 0.36 | 2.47 ± 0.38 | 2.54 ± 0.31 | 0.94 | 0.66 | 0.72 |

[1]Values are presented as LSM ± SE.

[2]Kidney weight was measured with one kidney.

**Supplemental Table S3.2: Relationship between metabolites and hormones in plasma of calves at birth (n=38)**

| Item[1] | Glucose | Lactate | Fructose | IGF-I | Leptin | Adiponectin | Insulin |
|---|---|---|---|---|---|---|---|
| Glucose | 1.00 | 0.27 | 0.16[2] | -0.17[2] | 0.02 | 0.03 | 0.32[§] |
| Lactate | 0.27 | 1.00 | -0.26[2] | -0.34[2*] | -0.19 | -0.20 | 0.03 |
| Fructose | 0.16 | -0.26 | 1.00 | 0.02[3] | 0.54[***] | 0.14 | 0.51[**] |
| IGF-I | -0.17 | -0.34[*] | 0.02[3] | 1.00 | 0.27 | 0.08 | 0.16 |
| Leptin | 0.02 | -0.19 | 0.54[2***] | 0.27[2] | 1.00 | 0.22 | 0.36[*] |
| Adiponectin | 0.03 | -0.20 | 0.14[2] | 0.08[2] | 0.22 | 1.00 | -0.15 |
| Insulin | 0.32[§] | 0.03 | 0.51[2**] | 0.16[2] | 0.36[*] | -0.15 | 1.00 |

[1]IGF-I = Insulin-like growth factor.

[2]n = 37.

[3]n = 36.

[*]$P < 0.05$; [**]$P < 0.01$; [***]$P < 0.001$.; [§]$P < 0.1$.

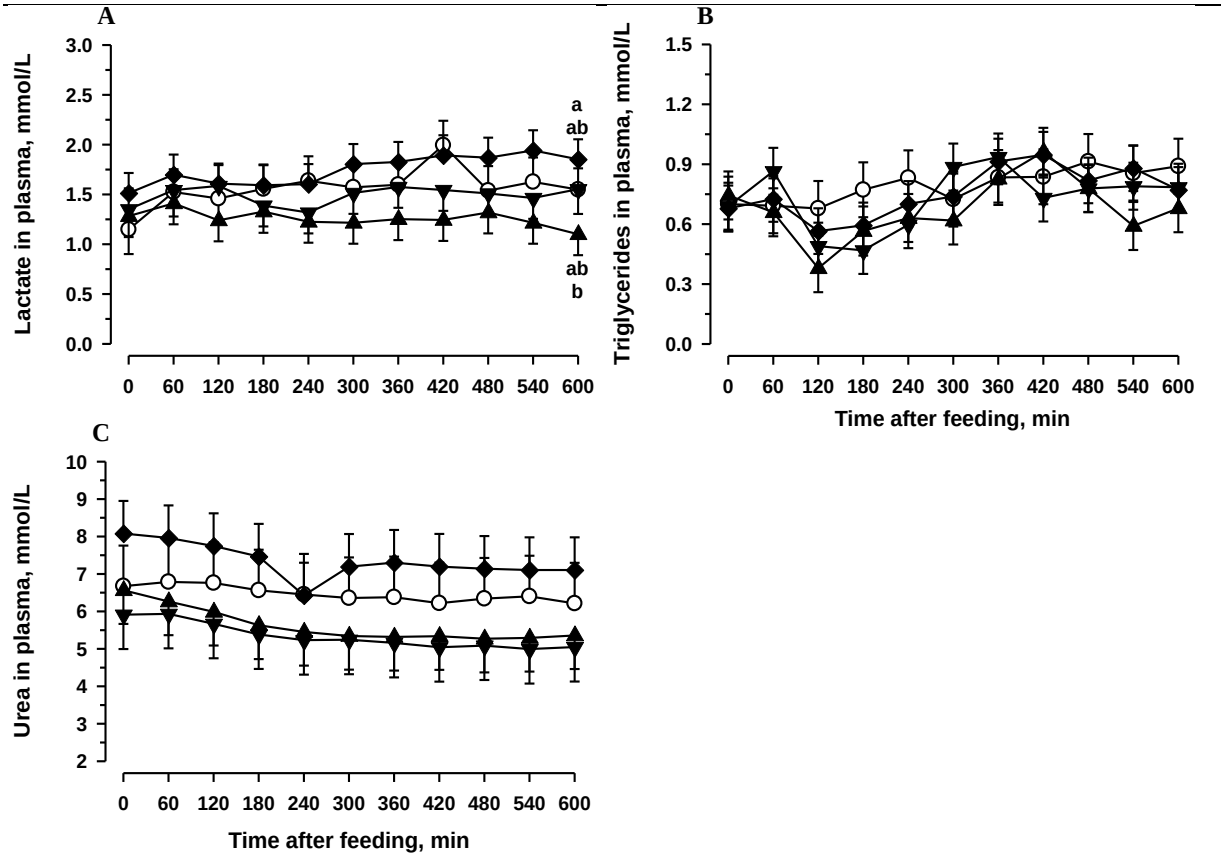
**Supplemental Table S3.3: Relationship between metabolites and hormones in plasma of dams and calves at birth (n=38)**

| | Variables in calf plasma | | | | | | |
|---|---|---|---|---|---|---|---|
| Variables in maternal plasma[1] | Glucose | Lactate | Fructose | IGF-I | Leptin | Adiponectin | Insulin |
| Glucose | 0.37[*] | 0.20 | 0.28[§] | 0.01 | 0.19 | -0.36[*] | 0.32[§] |
| IGF-I | 0.00 | -0.11 | 0.21[2] | 0.36[2*] | 0.16 | 0.12 | 0.04 |
| Leptin | -0.16 | -0.02 | -0.22[2] | 0.25[2] | -0.10 | -0.13 | 0.00 |
| Adiponectin | -0.16 | -0.06 | 0.10[2] | -0.03[2] | 0.07 | 0.03 | -0.09 |
| Insulin | -0.13 | -0.02 | 0.01[2] | 0.06[2] | 0.13 | -0.34[*] | 0.13 |

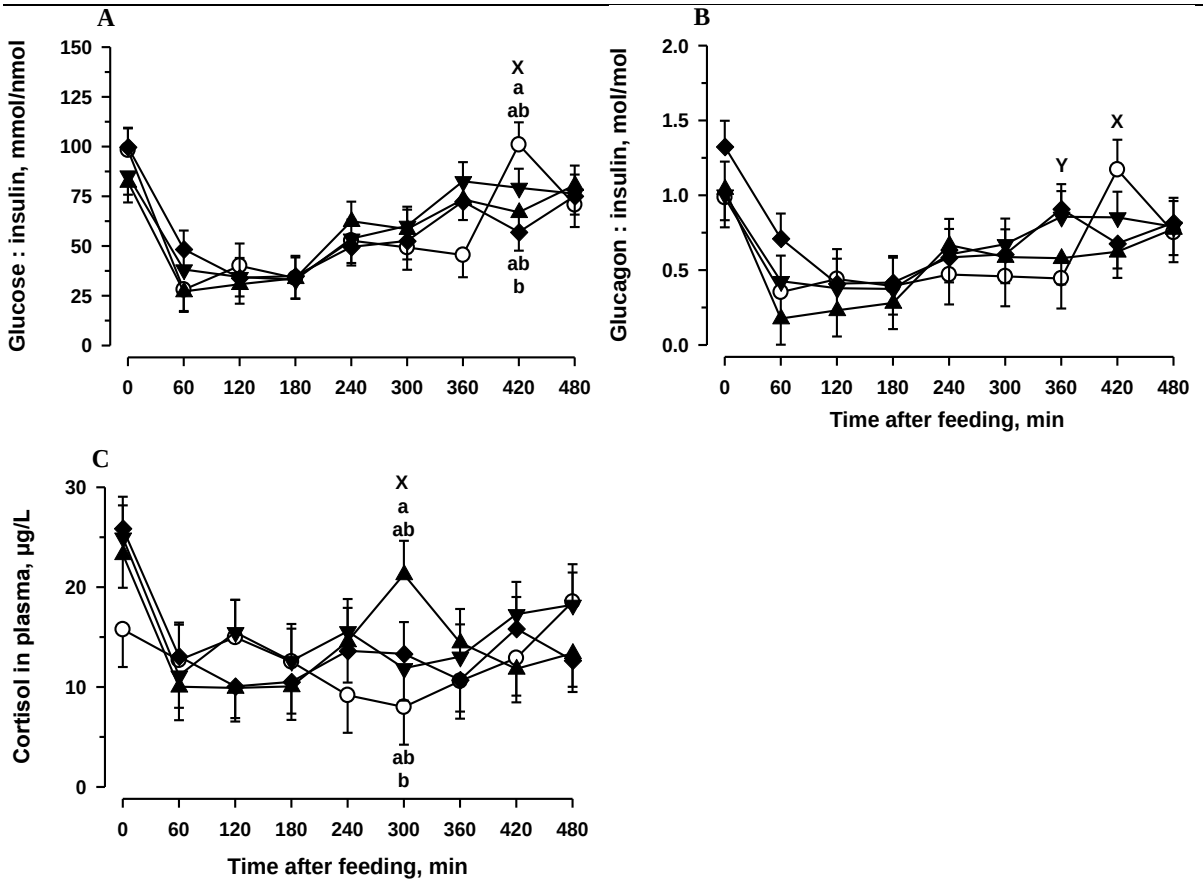
[1]IGF-I = Insulin-like growth factor.

[2]n = 37.

[*]$P < 0.05$; [§]$P < 0.1$.

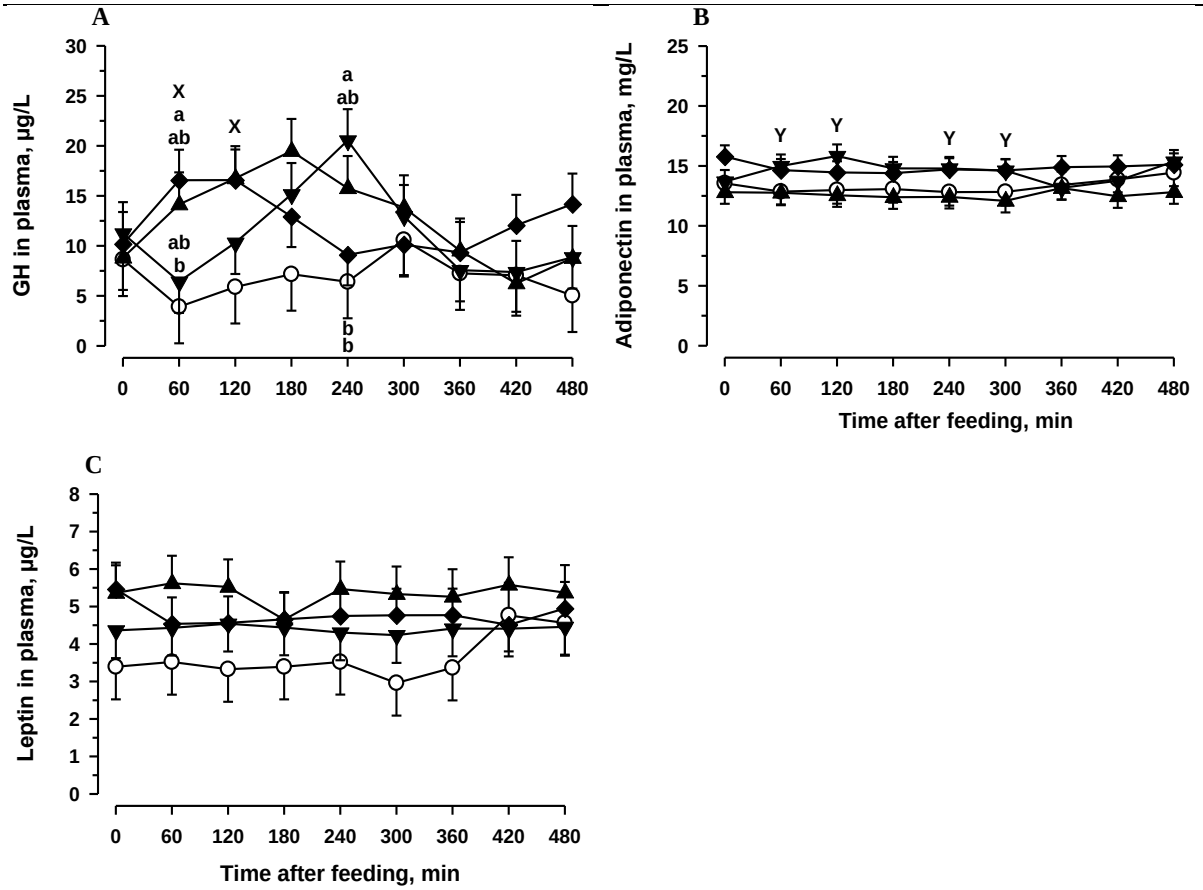



**Supplemental Figure S3.1: Postprandial concentrations of lactate (A), triglycerides (B), and urea (C) on d 4 of life in plasma of calves, whose dams were supplemented with coconut oil (CTRL; ○; n = 6), linseed and safflower oil (EFA; ▲; n = 7), Lutalin (CLA; *cis*-9, *trans*-11 and *trans*-10, *cis*-12; ▼; n = 8), or a combination of the EFA and CLA supplement (EFA+CLA; ♦; n = 8). Data are presented as LSM and SE. $^{a-b}$ Different superscripts represent significant differences among groups at the same time point ($P < 0.05$). Significant effect ($P < 0.05$) for lactate (time) and triglycerides (time and EFA × time interaction).**




**Supplemental Figure S3.2: Postprandial glucose/insulin ratio (A), glucagon/insulin ratio (B), and cortisol concentration (C) on d 4 of life in plasma of calves, whose dams were supplemented with coconut oil (CTRL; ○; n = 6), linseed and safflower oil (EFA; ▲; n = 7), Lutalin (CLA; *cis*-9, *trans*-11 and *trans*-10, *cis*-12; ▼; n = 8), or a combination of the EFA and CLA supplement (EFA+CLA; ♦; n = 8). Data are presented as LSM and SE.**

**[a-b] Different superscripts represent significant differences among groups at the same time point ($P < 0.05$). X indicates significant differences between EFA and non-EFA treated animals; Y indicates significant differences between CLA and non-CLA treated animals. Significant effect ($P < 0.05$) for glucose/insulin ratio (time, EFA × time, and CLA × time interaction), glucagon/insulin ratio (time, EFA × time, and CLA × time interaction), and cortisol (time).**




**Supplemental Figure S3.3: Postprandial concentrations of growth hormone (GH; A), adiponectin (B), and leptin (C) on d 4 of life in plasma of calves, whose dams were supplemented with coconut oil (CTRL; ○; n = 6), linseed and safflower oil (EFA; ▲; n = 7), Lutalin (CLA; *cis*-9, *trans*-11 and *trans*-10, *cis*-12; ▼; n = 8), or a combination of the EFA and CLA supplement (EFA+CLA; ♦; n = 8). Data are presented as LSM and SE. [a-b] Different superscripts represent significant differences among groups at the same time point ($P < 0.05$). X indicates significant differences between EFA and non-EFA treated animals; Y indicates significant differences between CLA and non-CLA treated animals. Significant effect ($P < 0.05$) for GH (EFA × time interaction) and adiponectin (CLA).**

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